

Supplemental Table and Figure Legends for

"In silico assessment of primers for eDNA studies using PrimerTree and application to characterize the biodiversity surrounding the Cuyahoga River"

Cannon MV, Hester J, Shalkhauser A, Chan ER, Logue K, Small ST and Serre D

Supplemental Table 1: Sample descriptions

Supplemental Table 2: Primer sequences

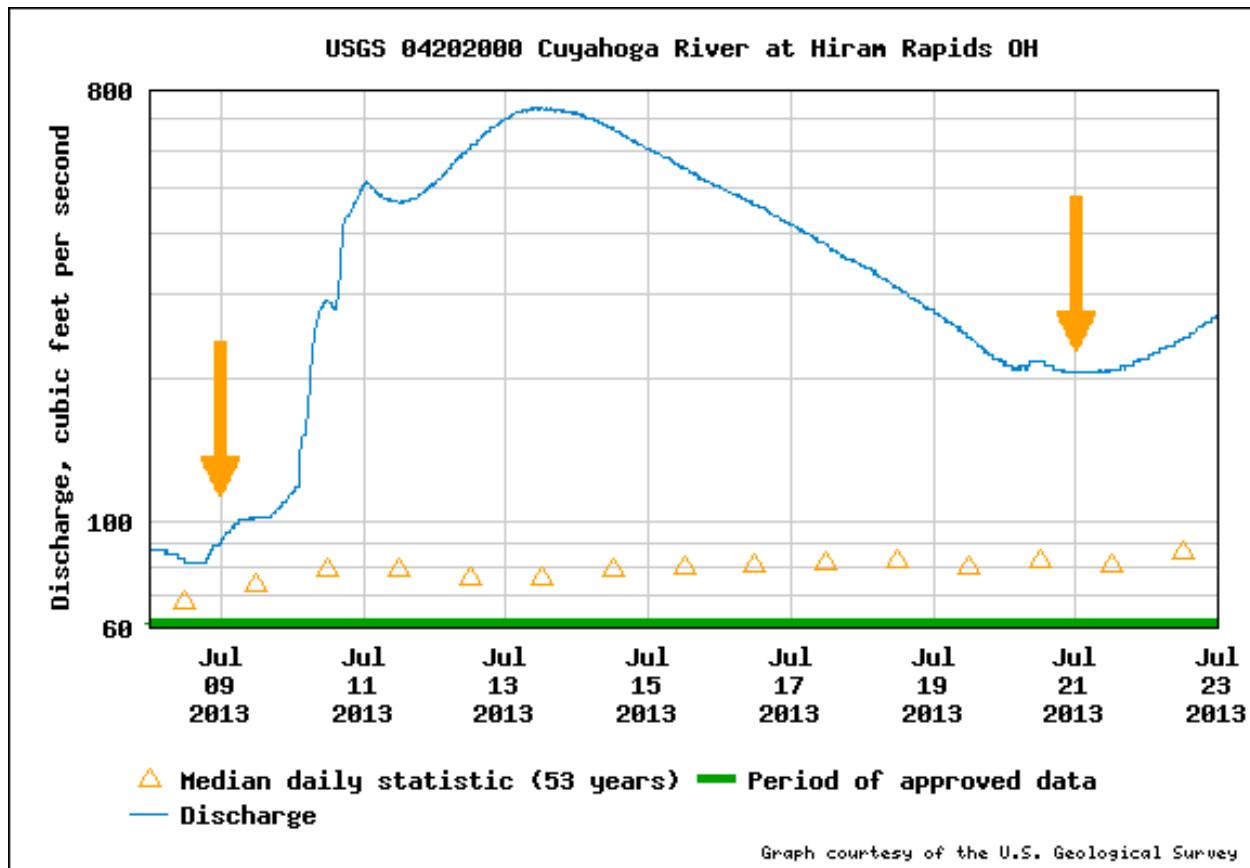
Supplemental Table 3: Primer length and specificity based on observed and *in silico* data (in brackets)

Supplemental Table 4: Taxonomy and raw read counts for vascular plant and bryophyte primers

Supplemental Table 5: Fish species identified using the mammalian 16S rRNA and fish mt-Cytb primers

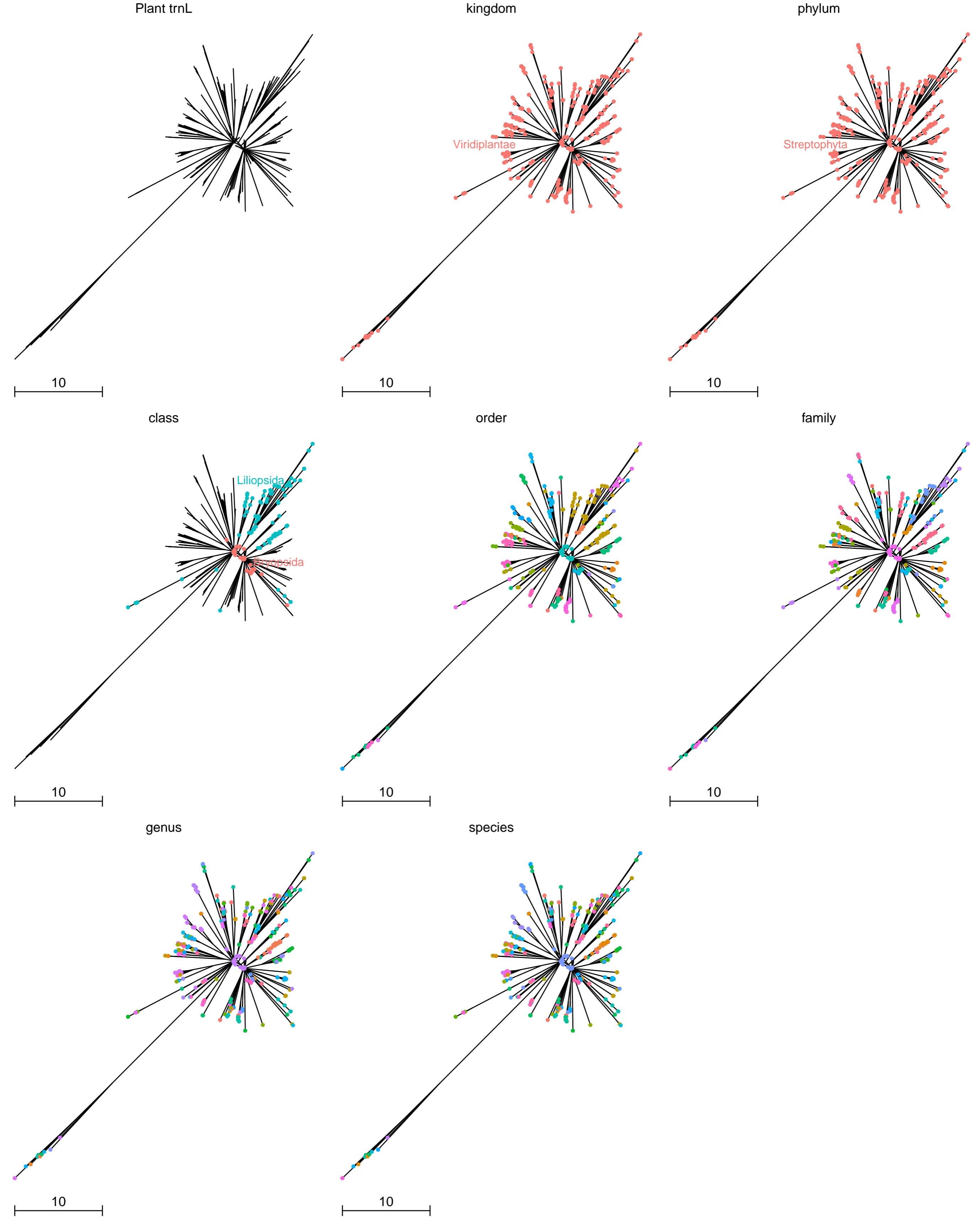
Supplemental Table 6: Raw read counts and species absence/presence calls for macroorganisms

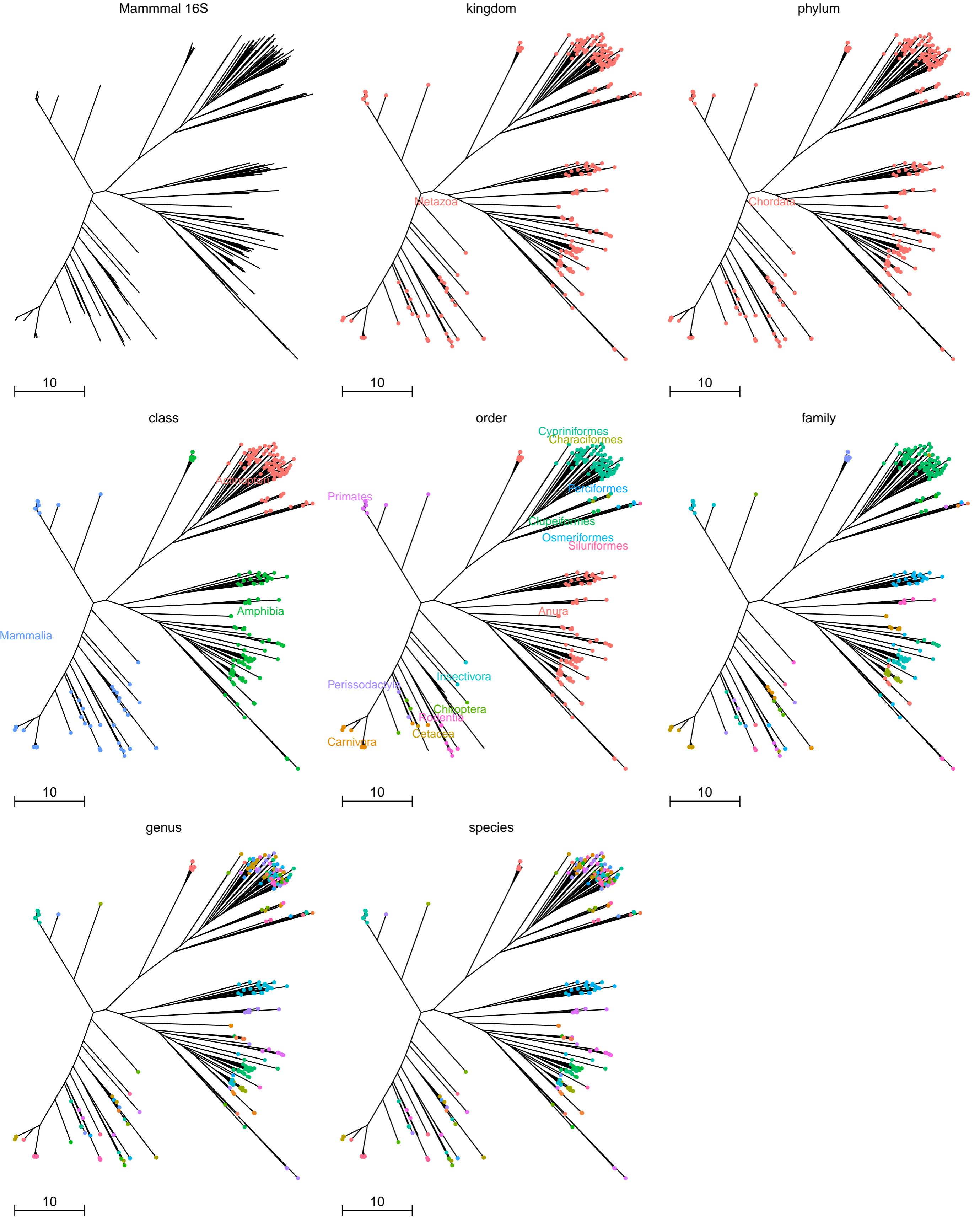
Supplemental Table 7: Experimental expense summary

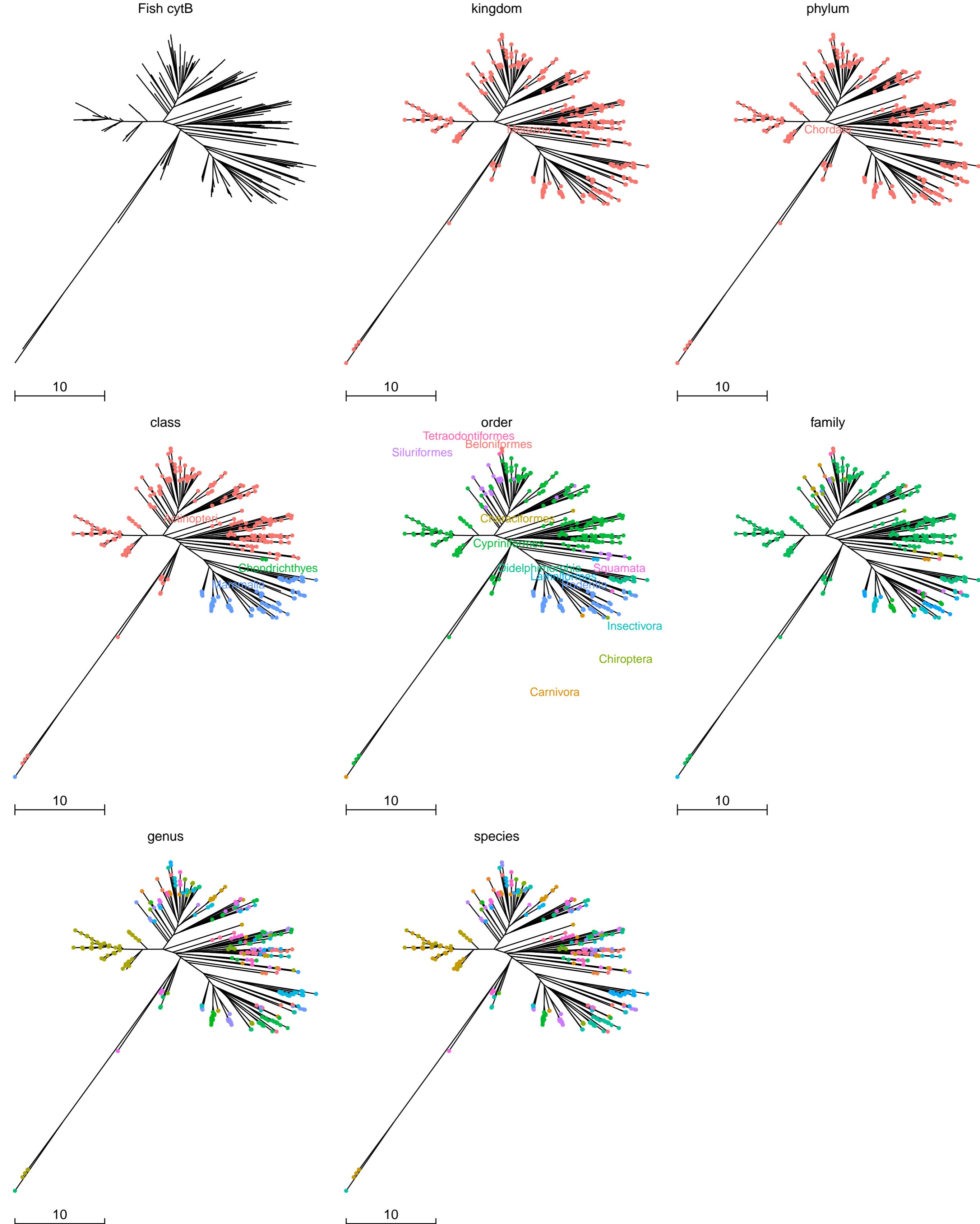


Supplemental Figure 1: USGS discharge at the days of sample collection. The figure shows the discharge of the Cuyahoga River at Hiram Rapids (Upper Cuyahoga, OH) in cubic feet per second (y-axis, in log scale) between July 9 and July 24 2013. The days of collection are indicated by the orange arrows.

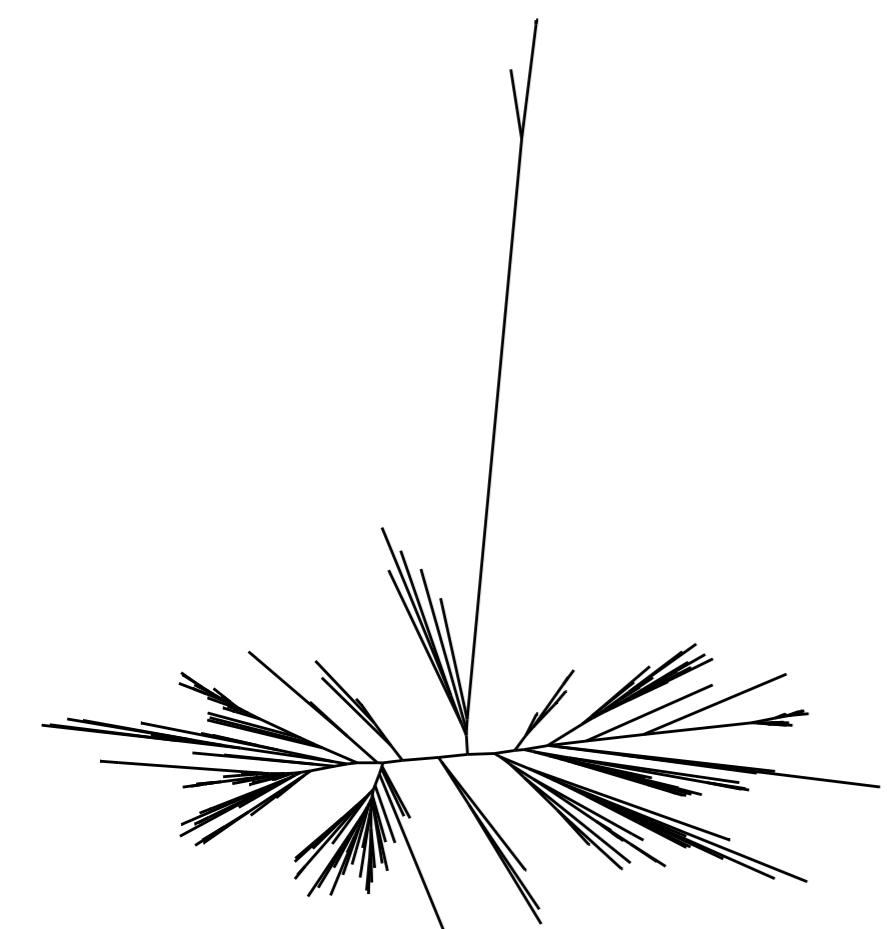
Supplemental Figure 2: PrimerTree results for the 12 primer pairs. Each primer set is denoted at the top of the page. Each page is divided into eight sections that show different taxonomic levels, with the points colored according to the taxa within that level. Levels with few taxa have colored text labels. The guide below each tree shows the scale in number of nucleotide differences.



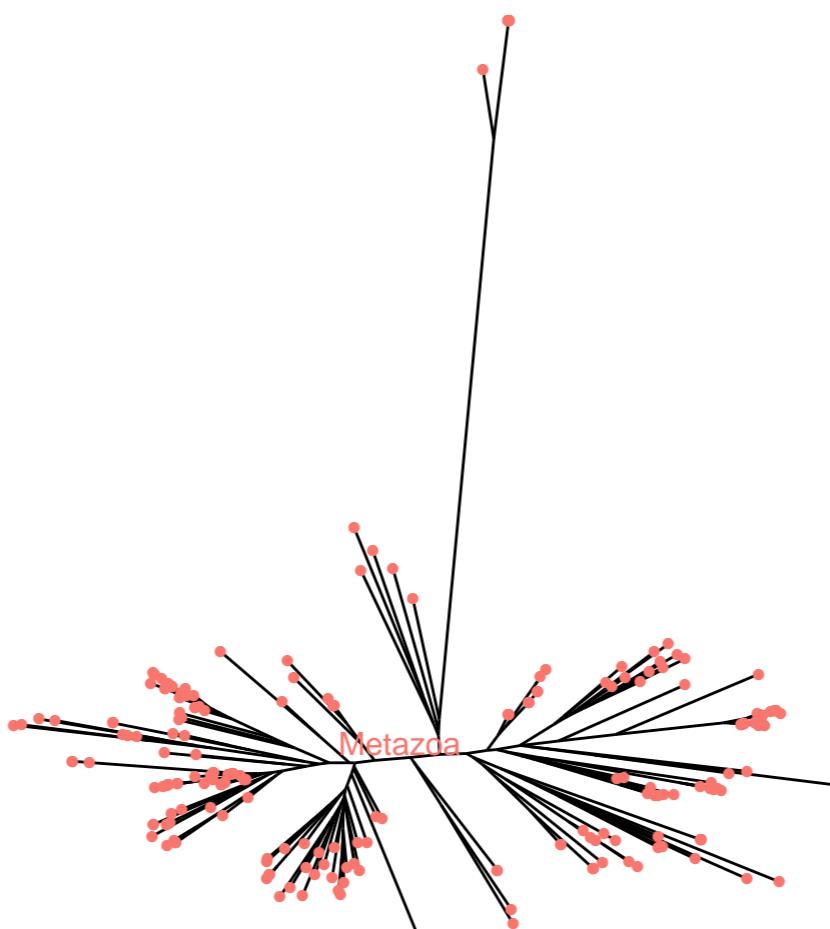




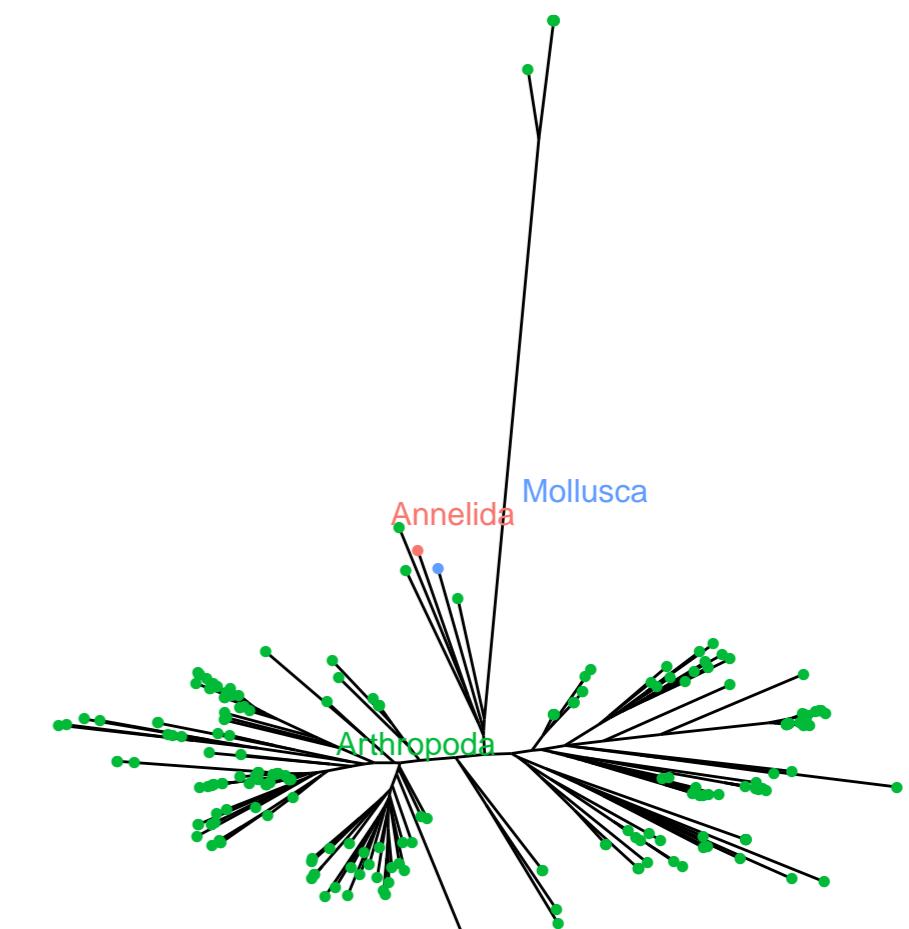
Insect COI_ZBJ_Art



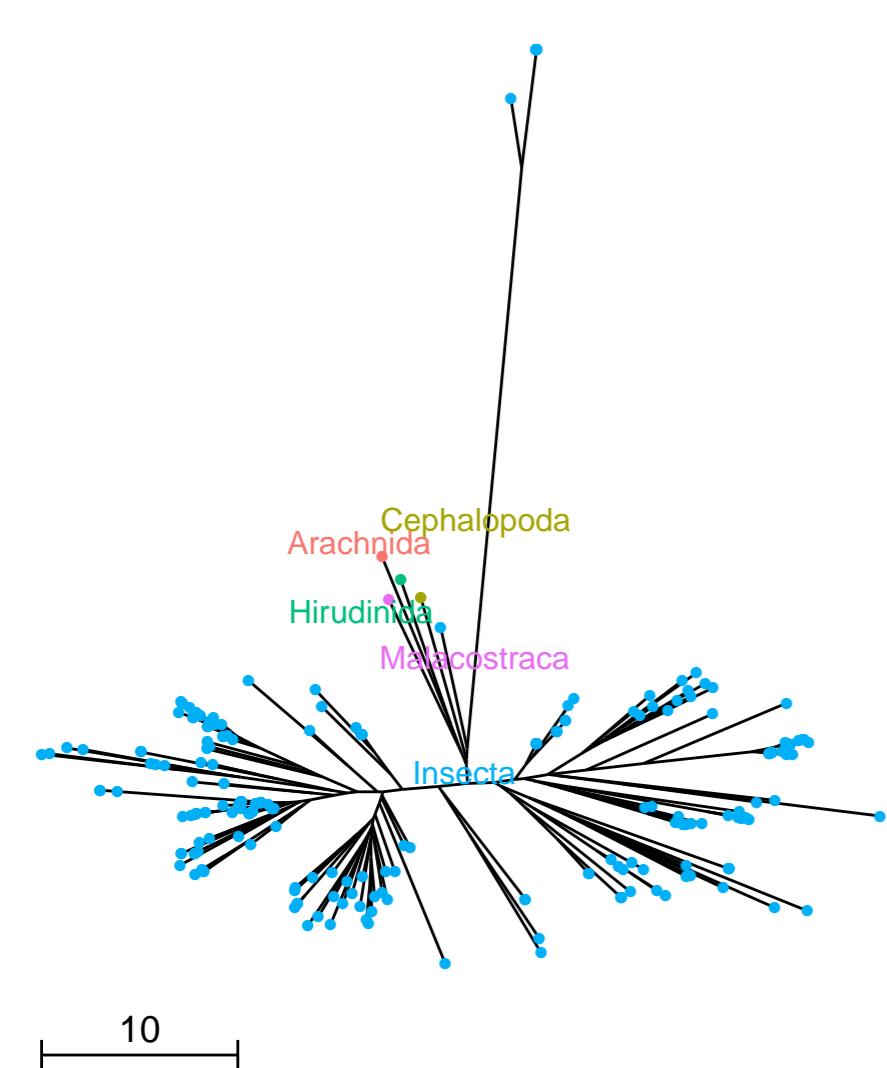
kingdom



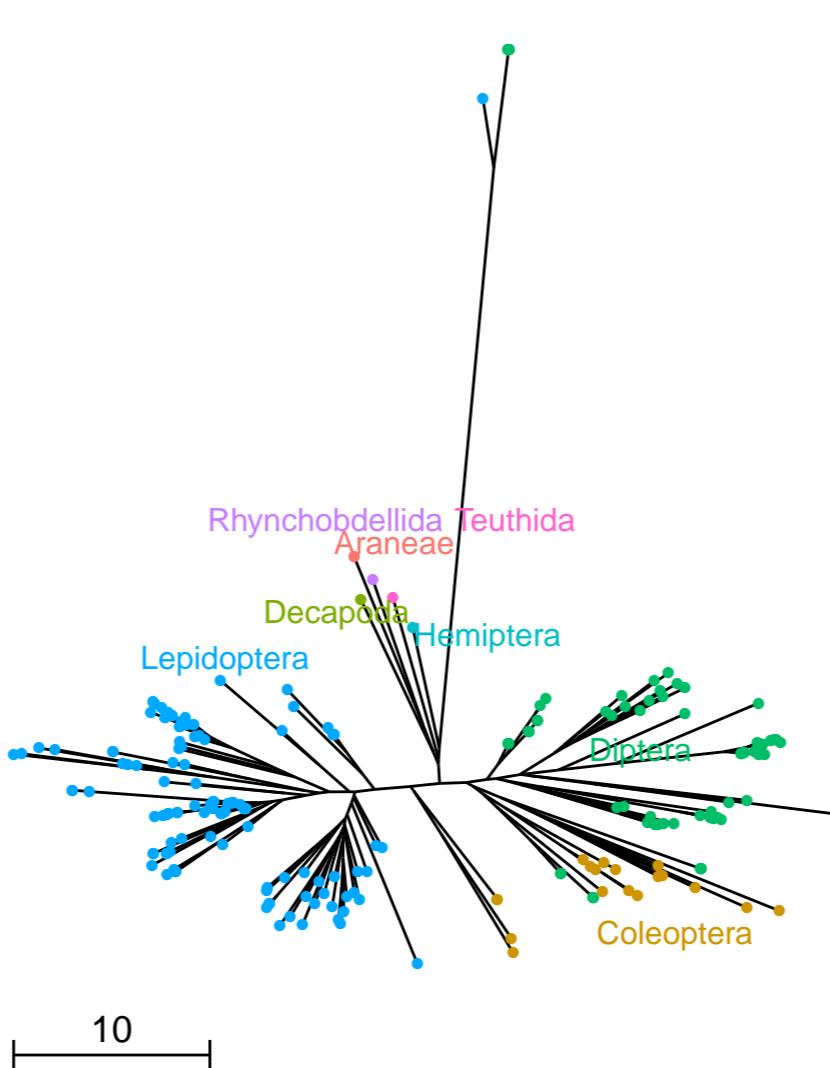
phylum



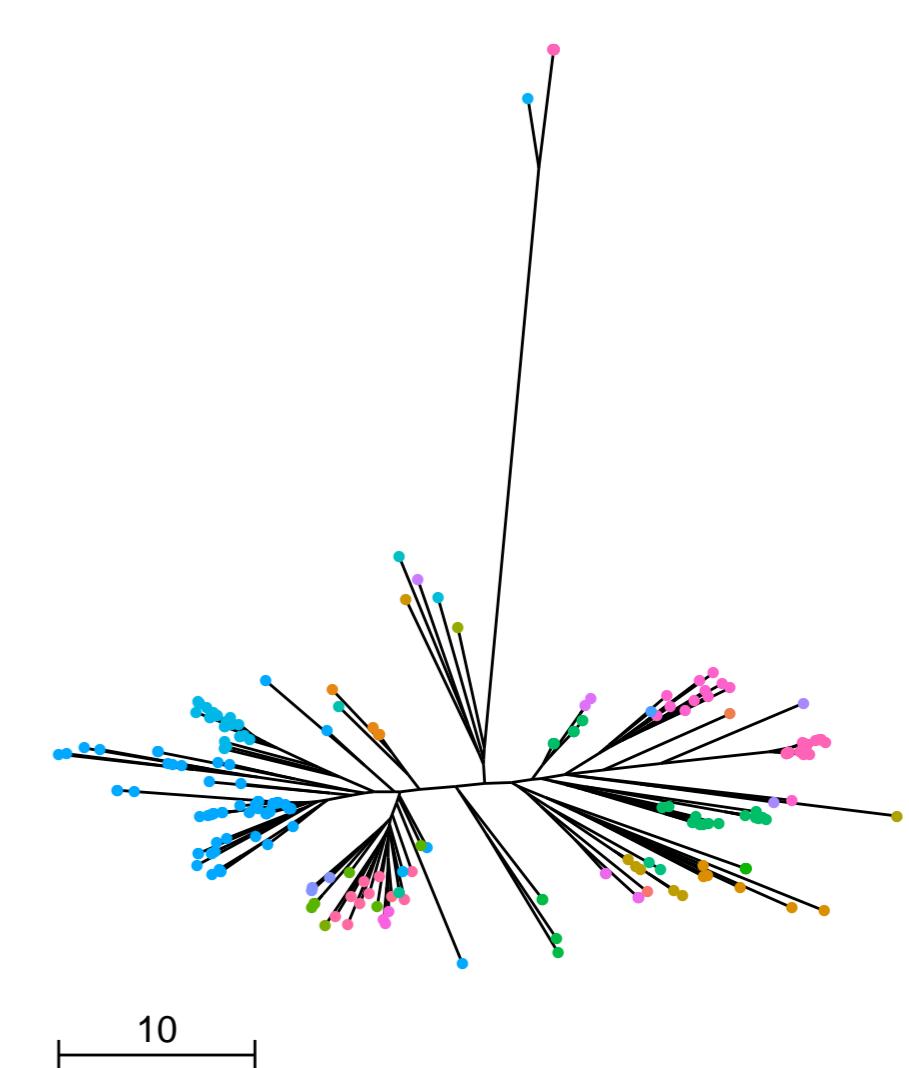
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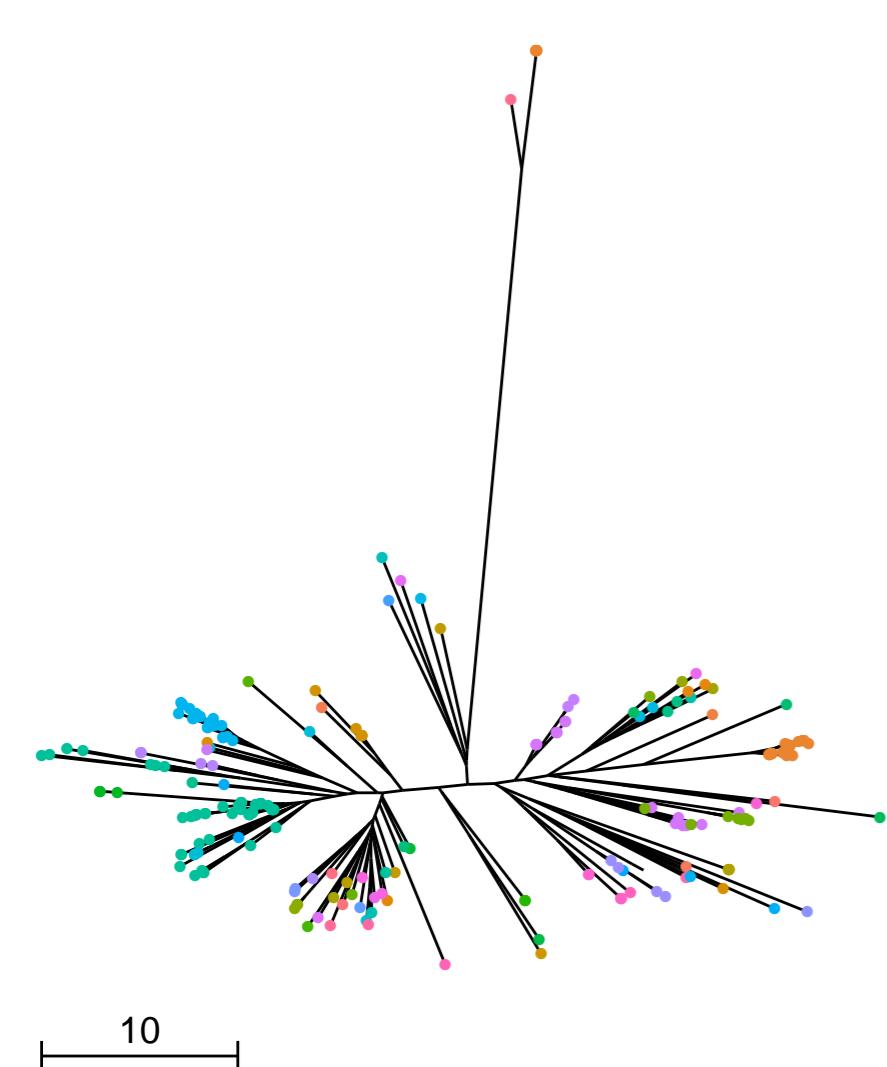
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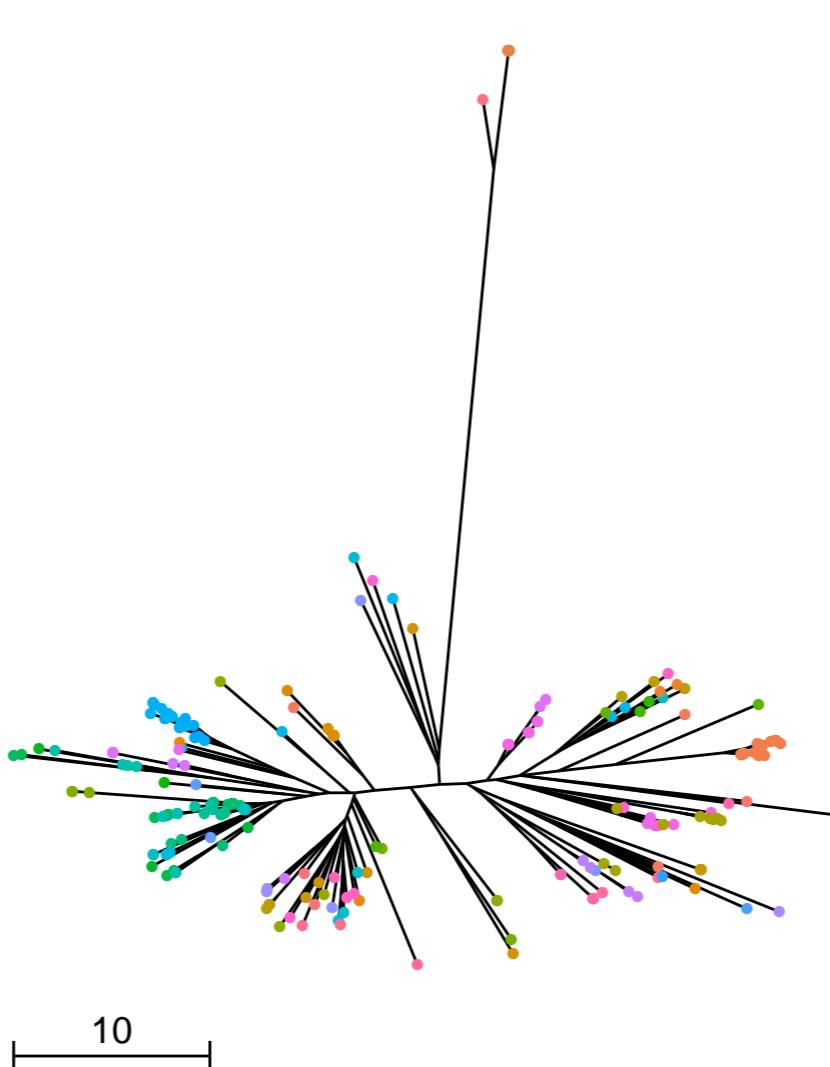
family

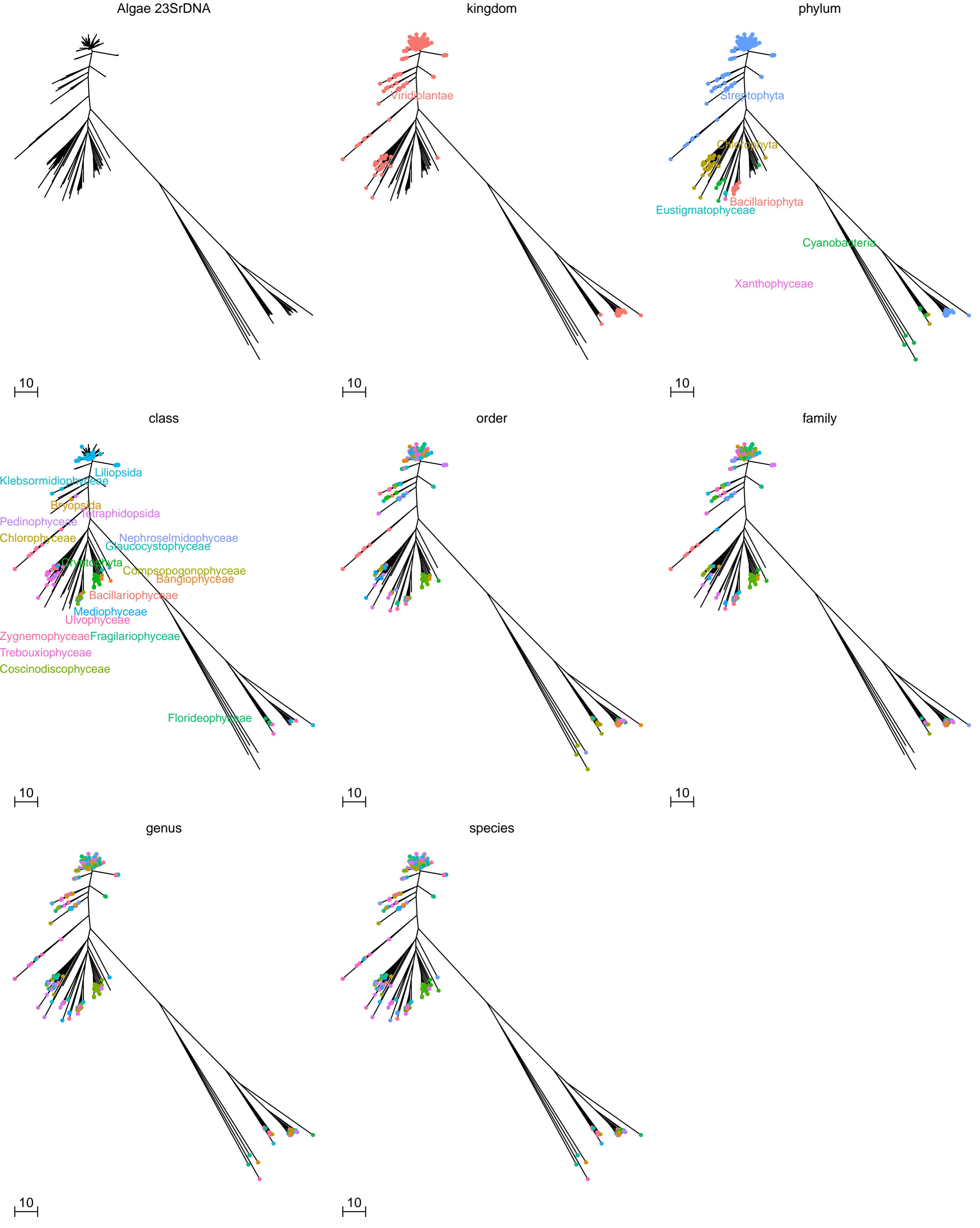


genus

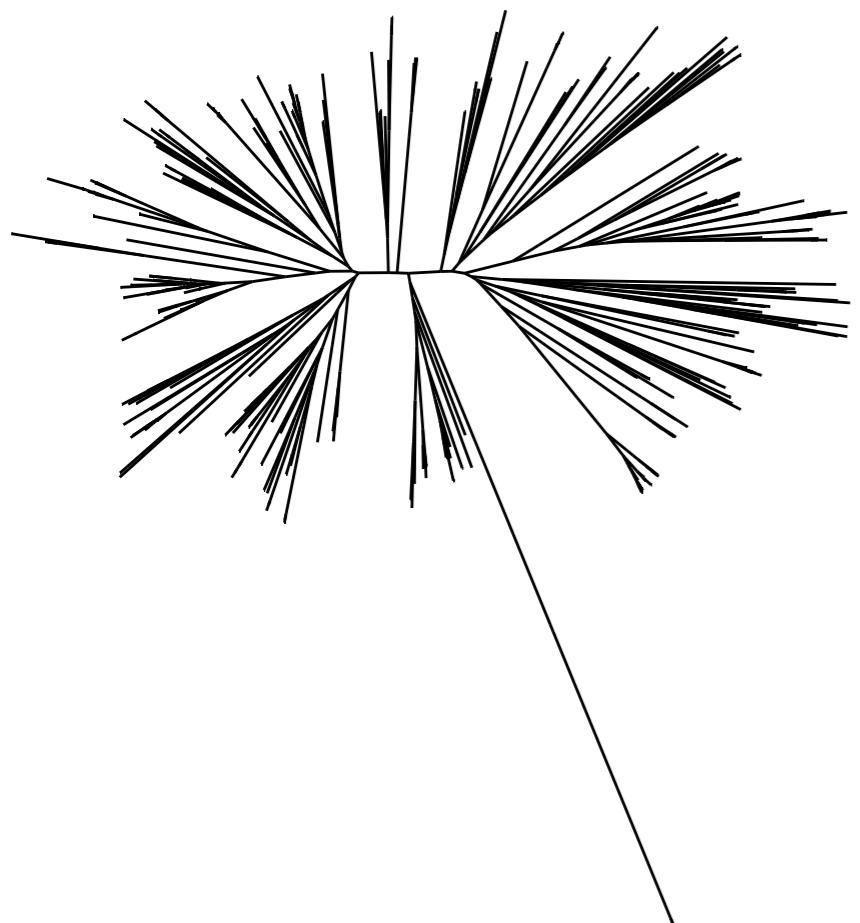


species

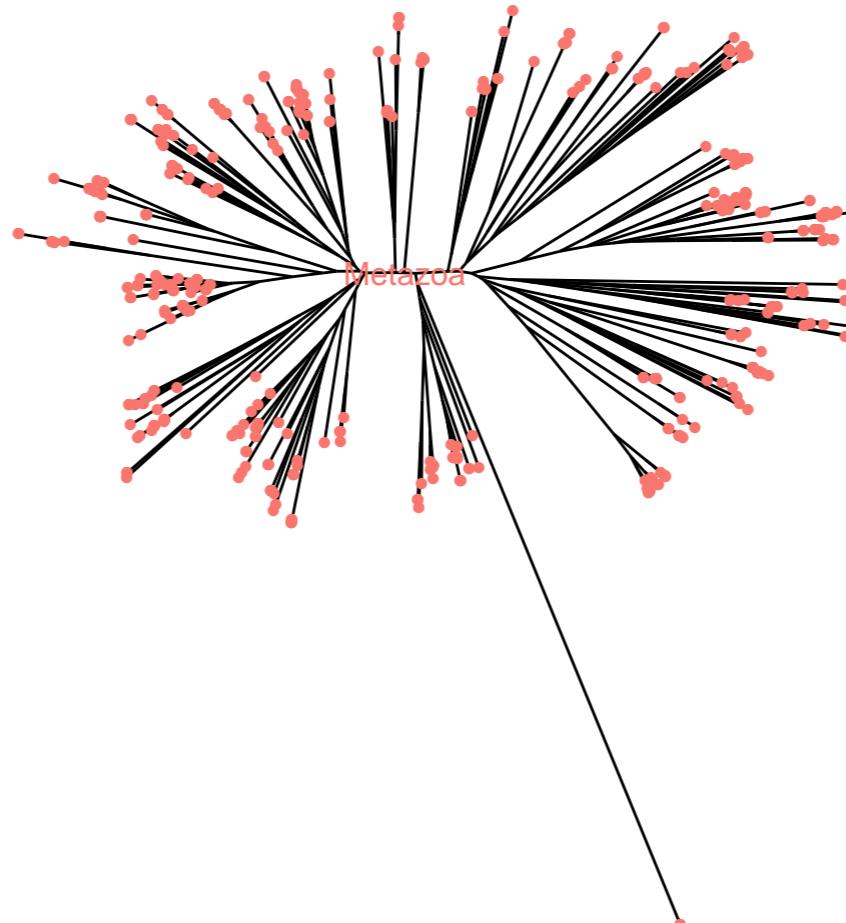




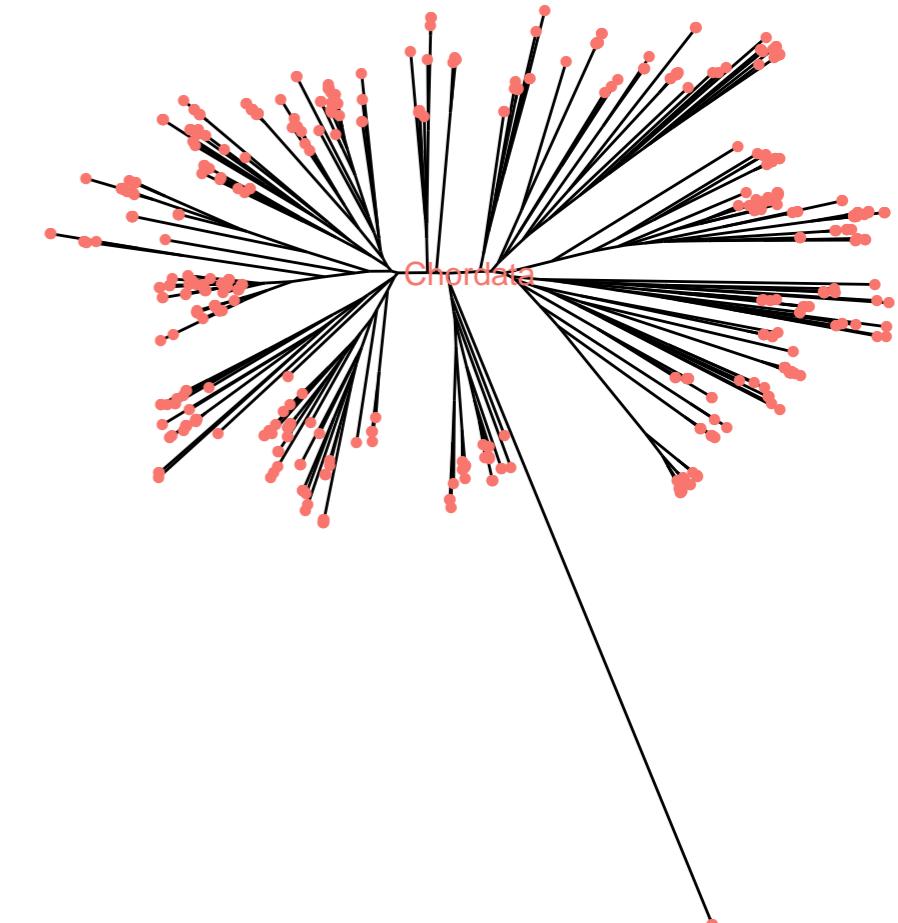
Amphibian cytB



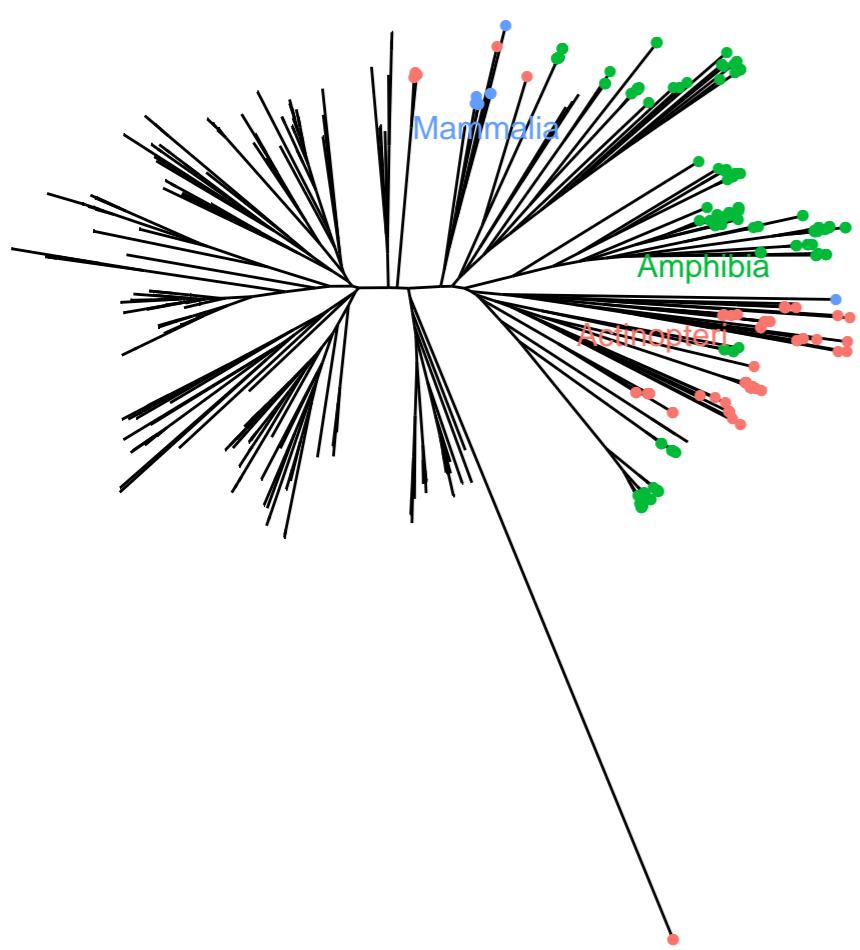
kingdom



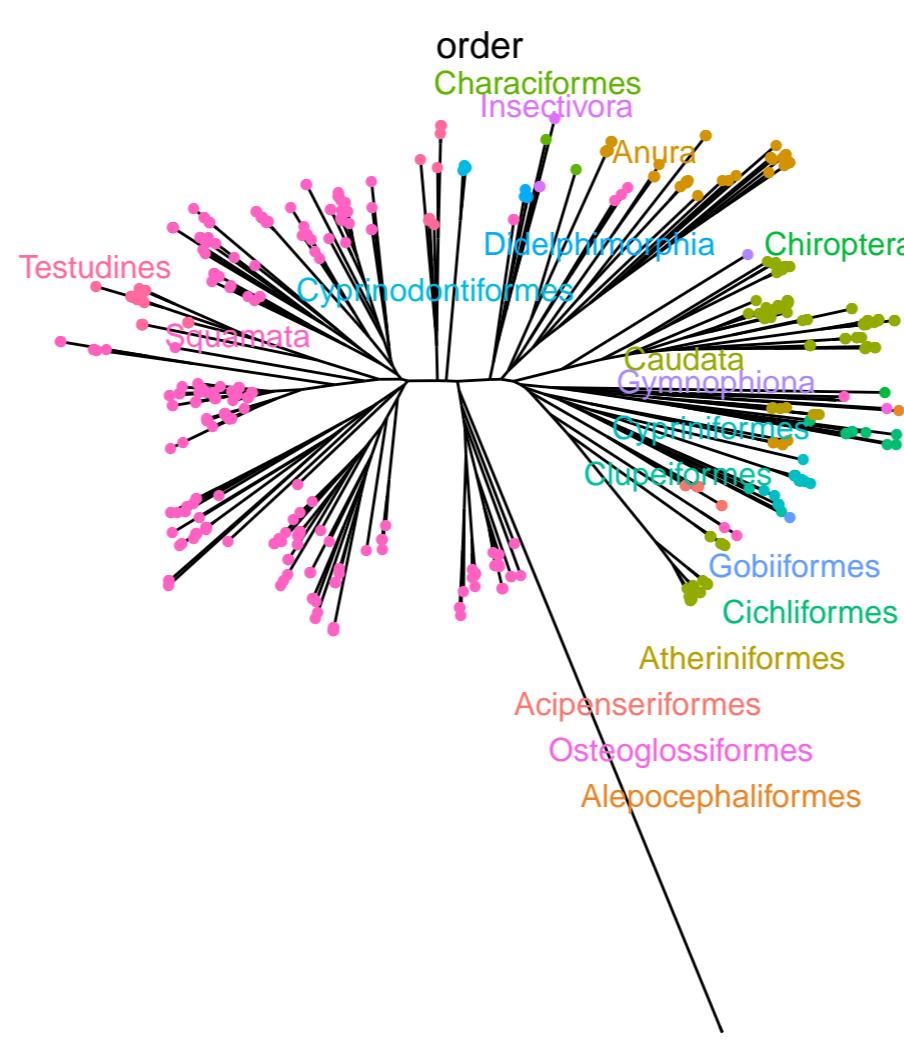
phylum



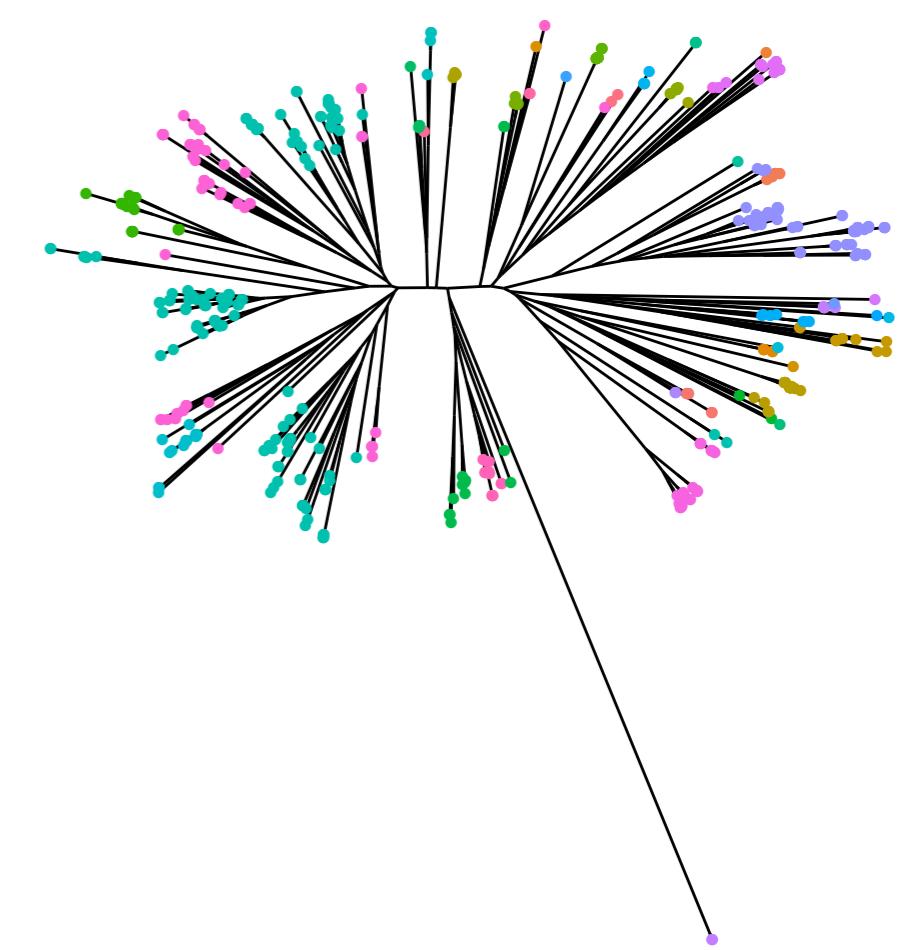
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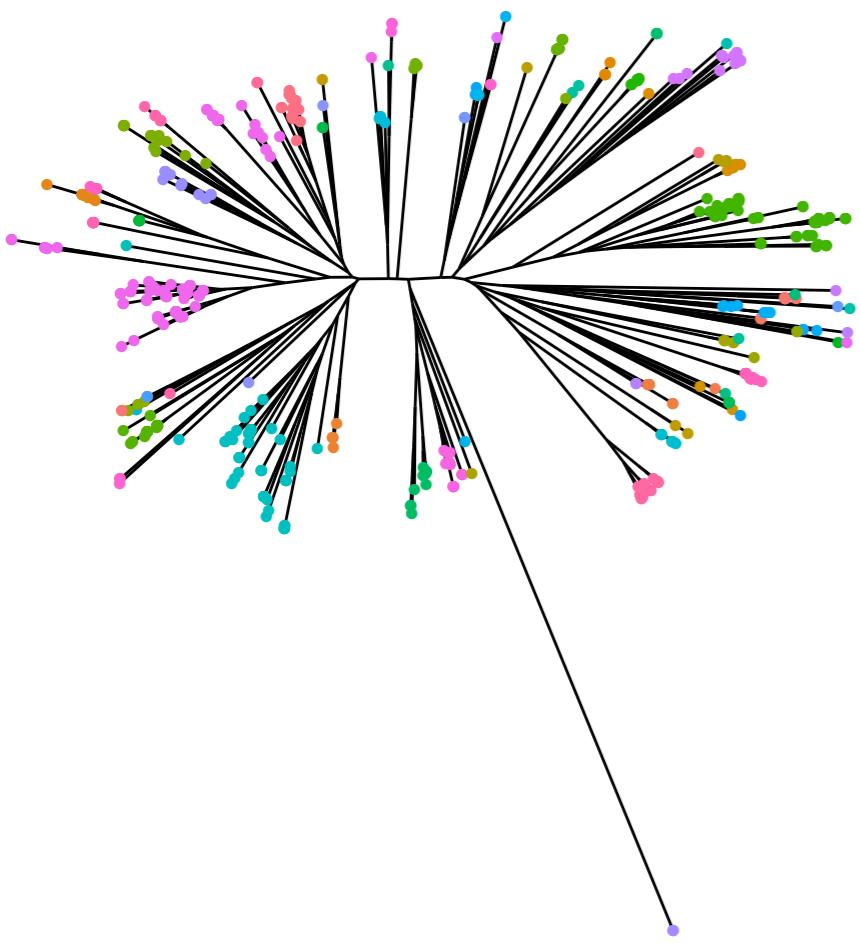
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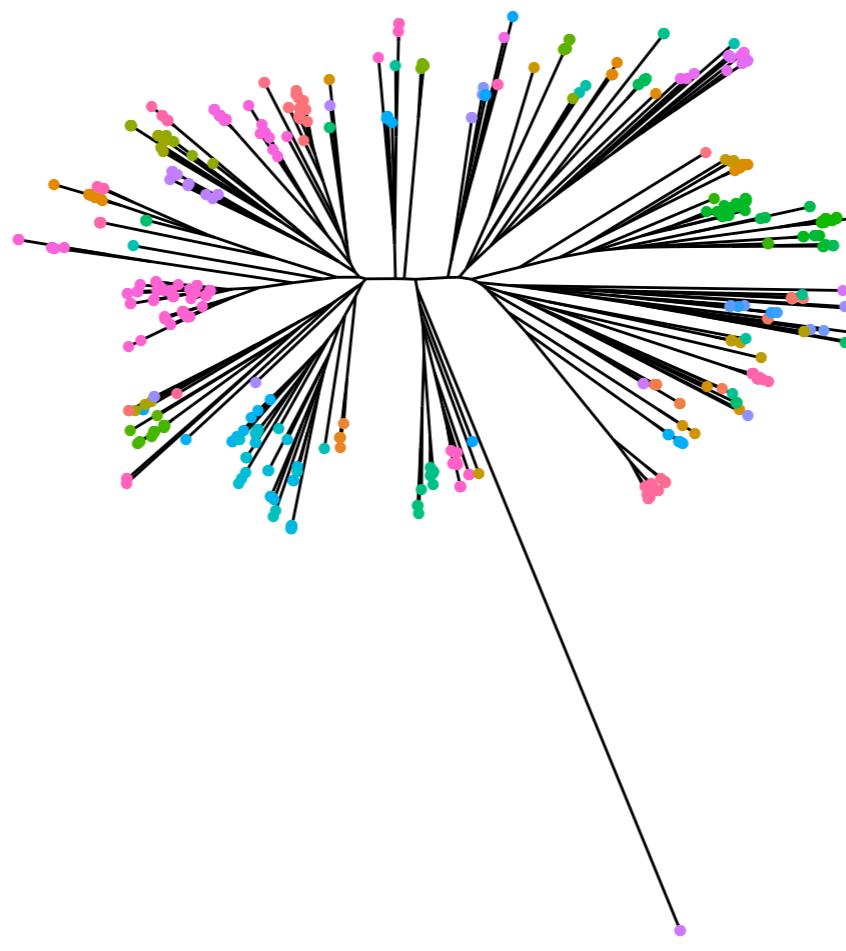
family



genus



species

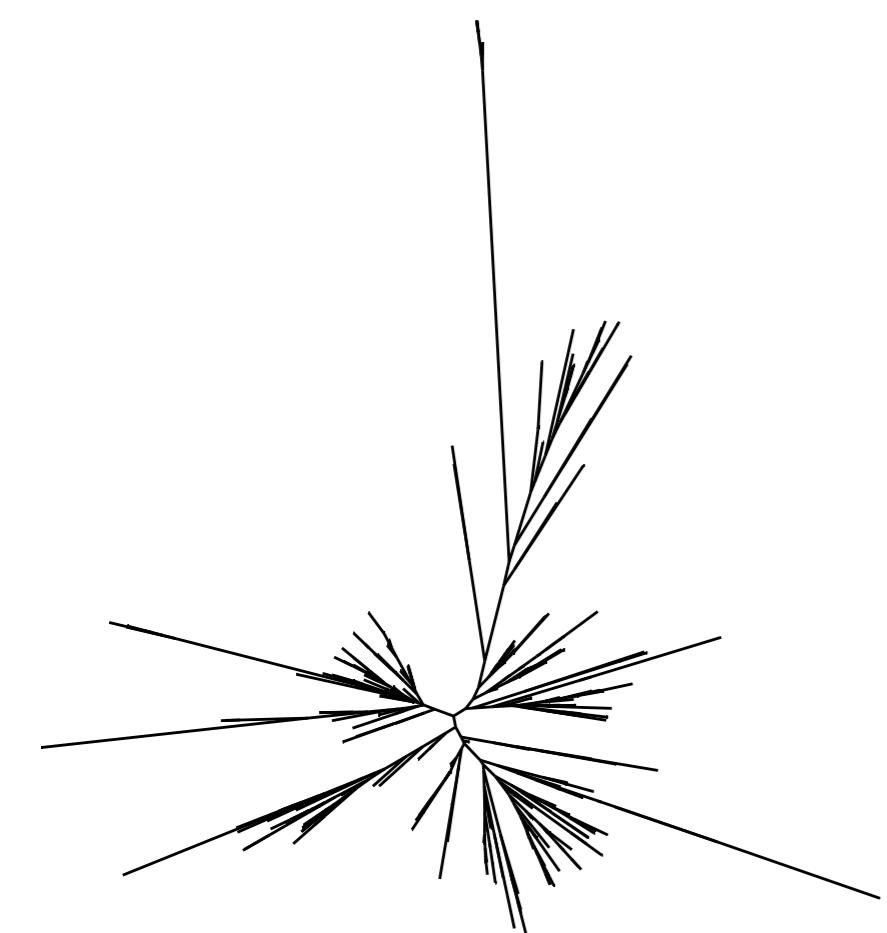


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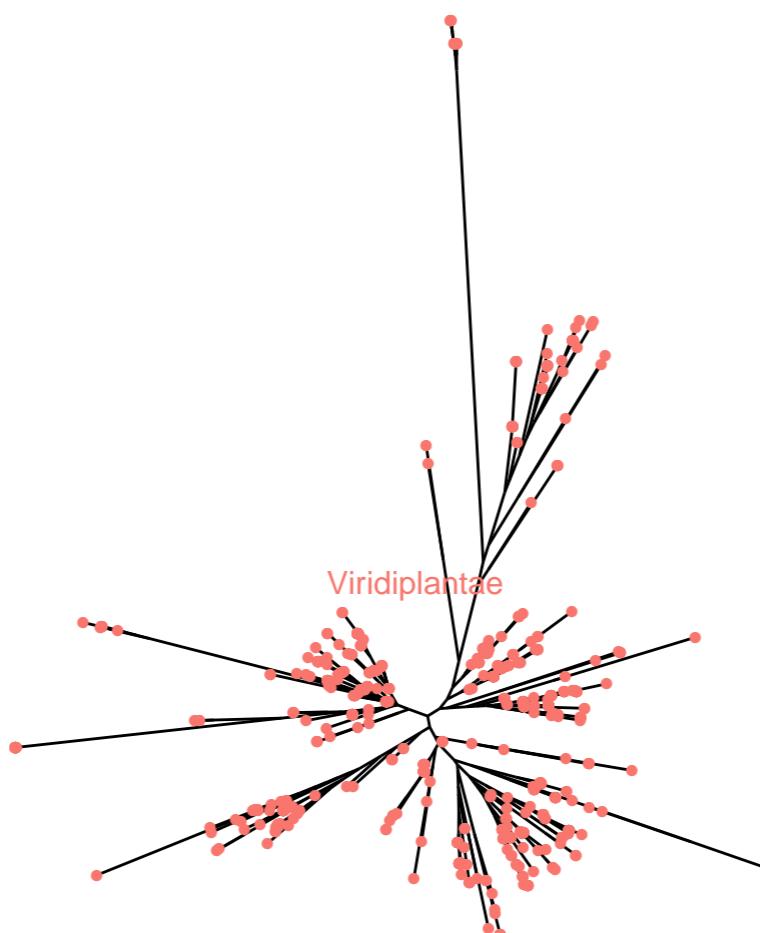
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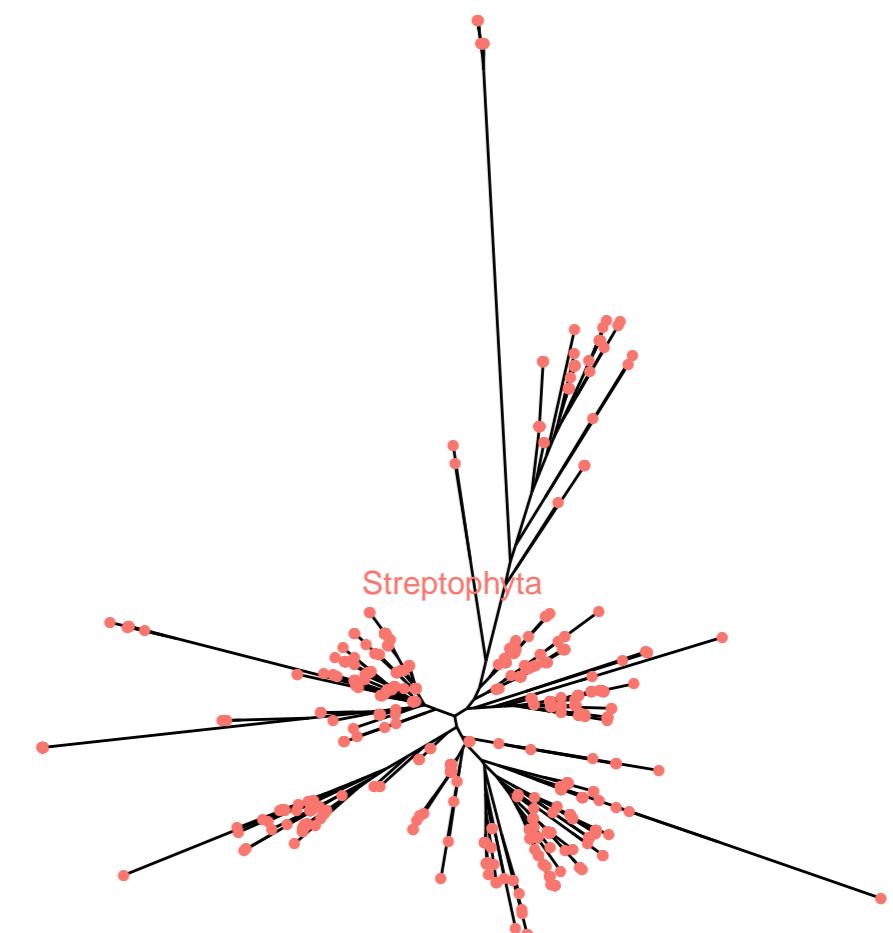


Bryophyte *trnL*

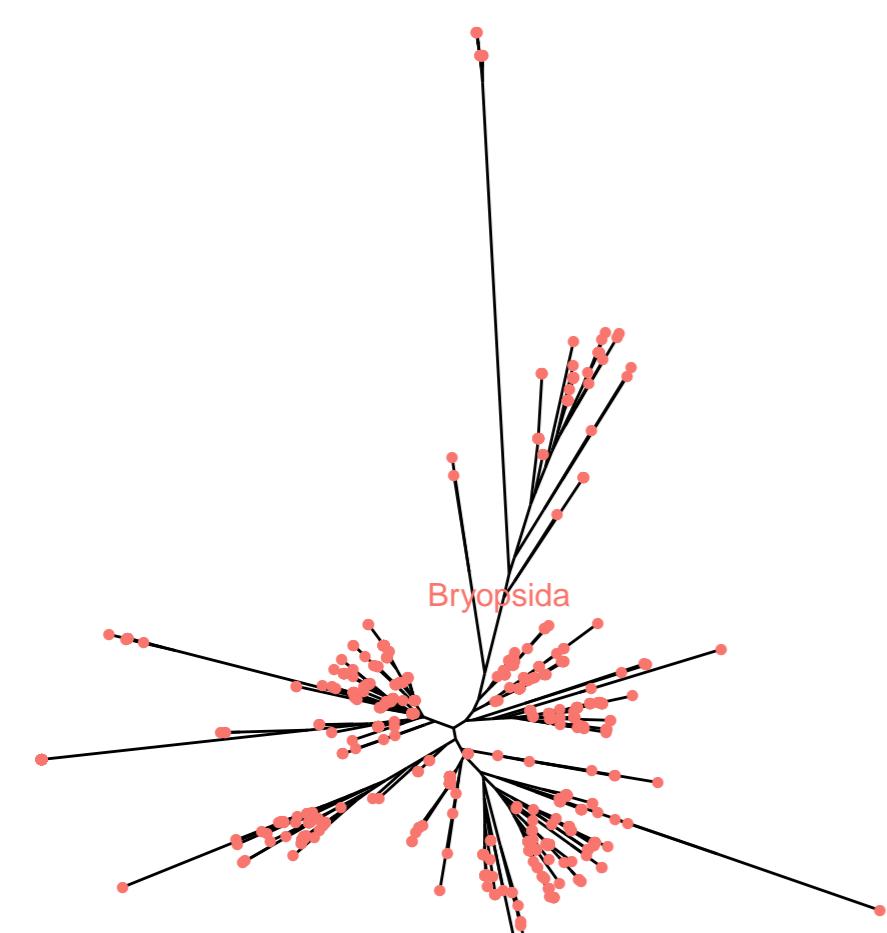
kingdom



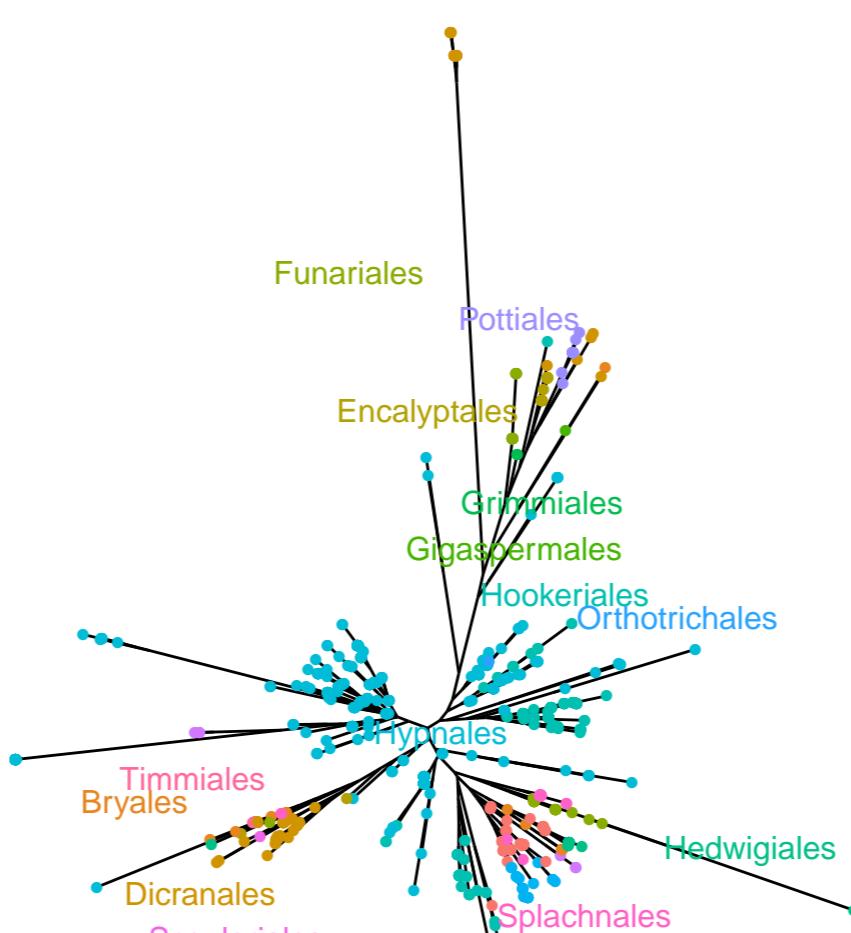
phylum



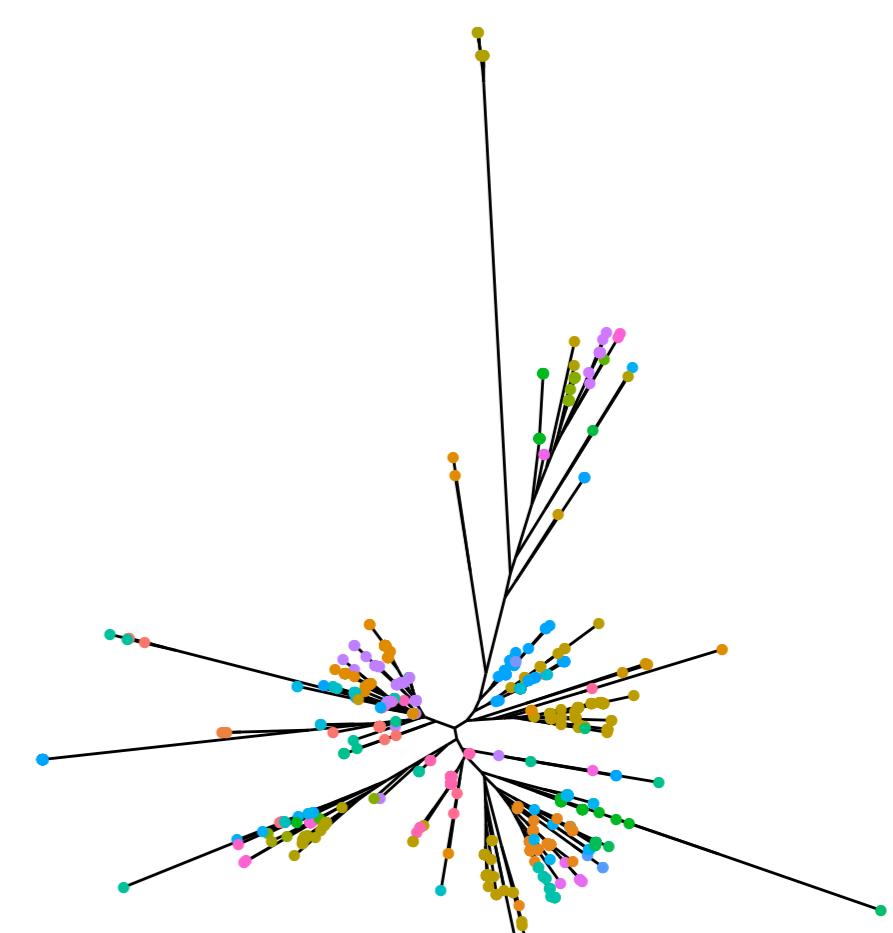
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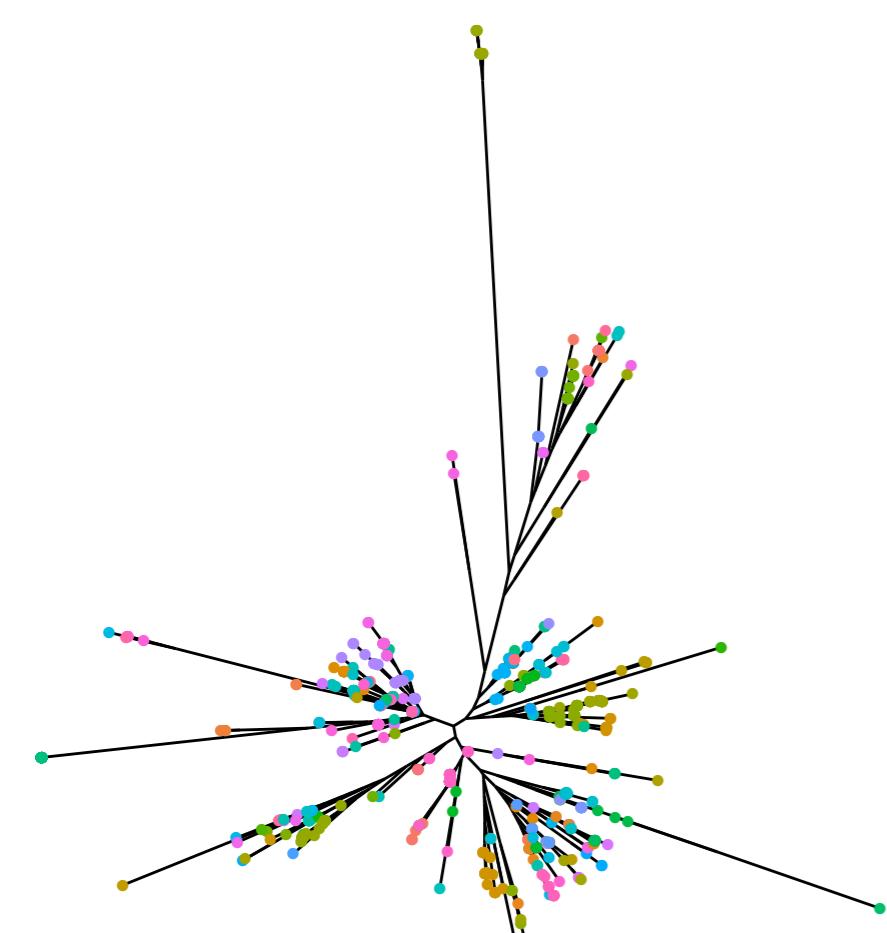
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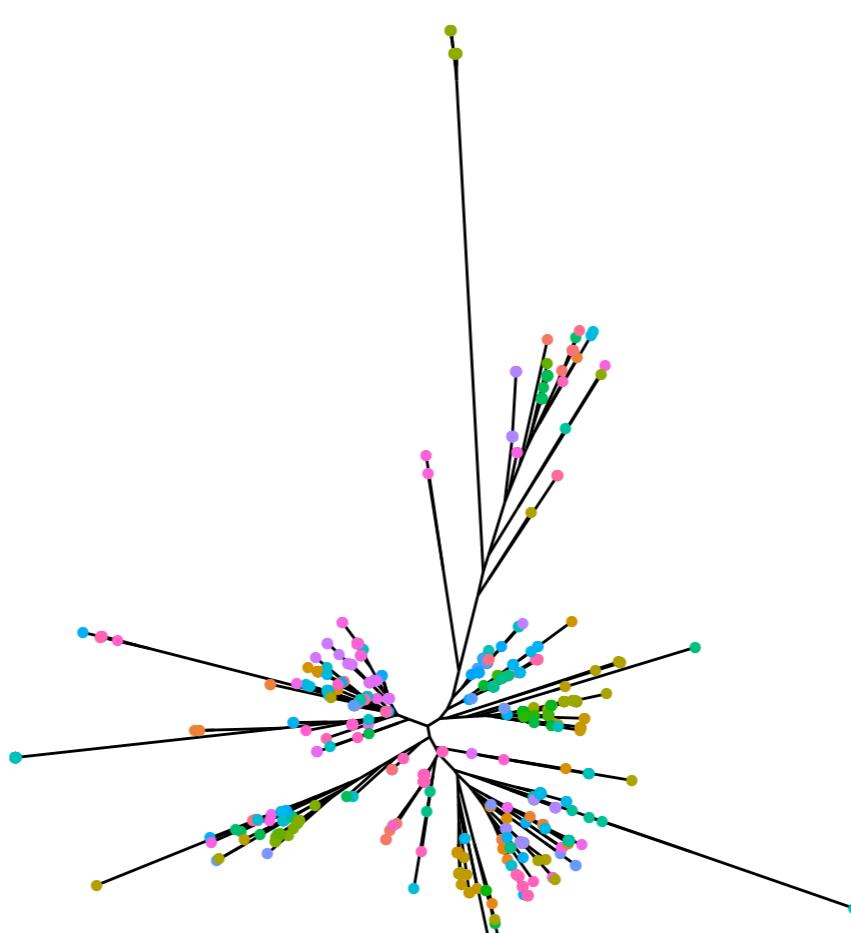
family



genus



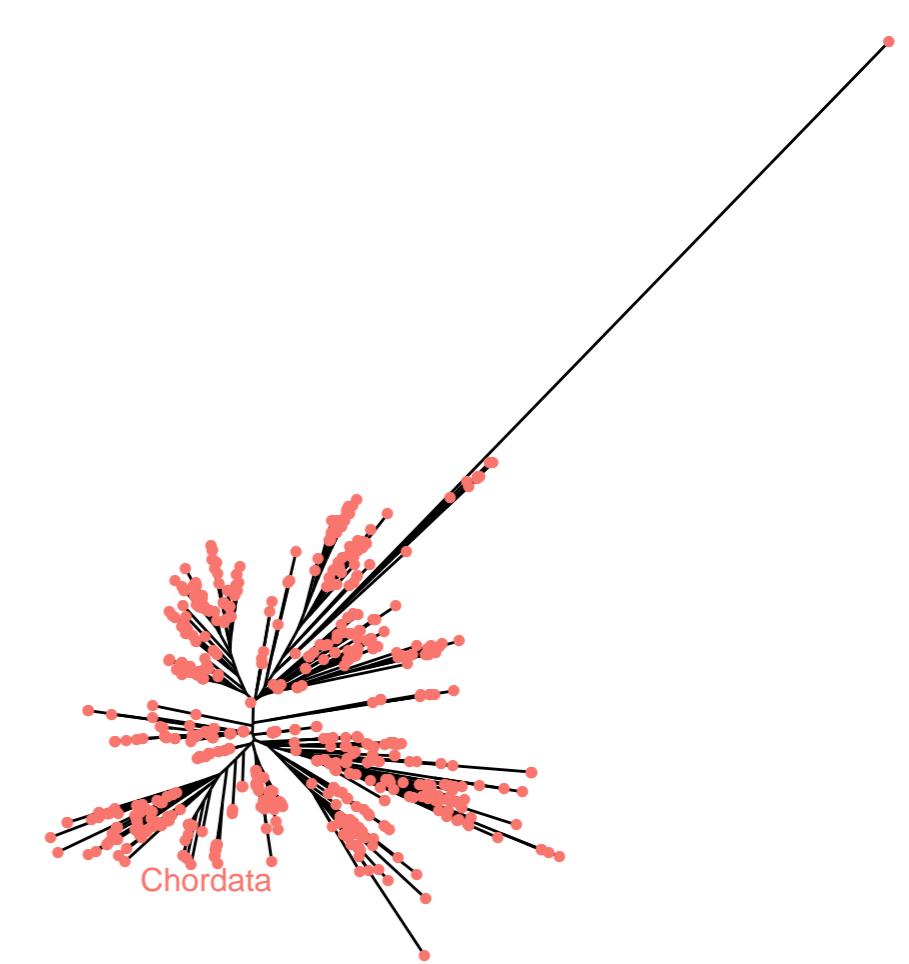
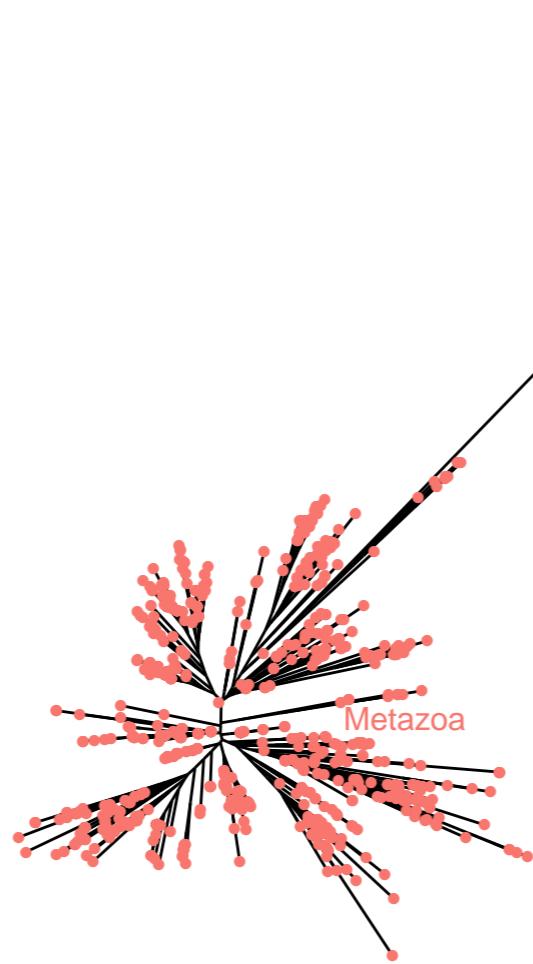
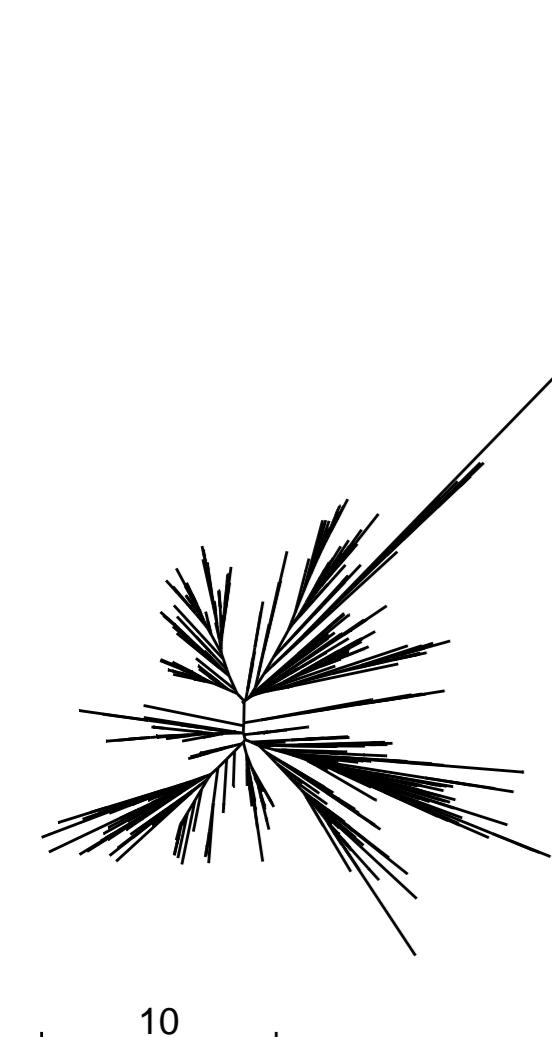
species



Aves 12S

kingdom

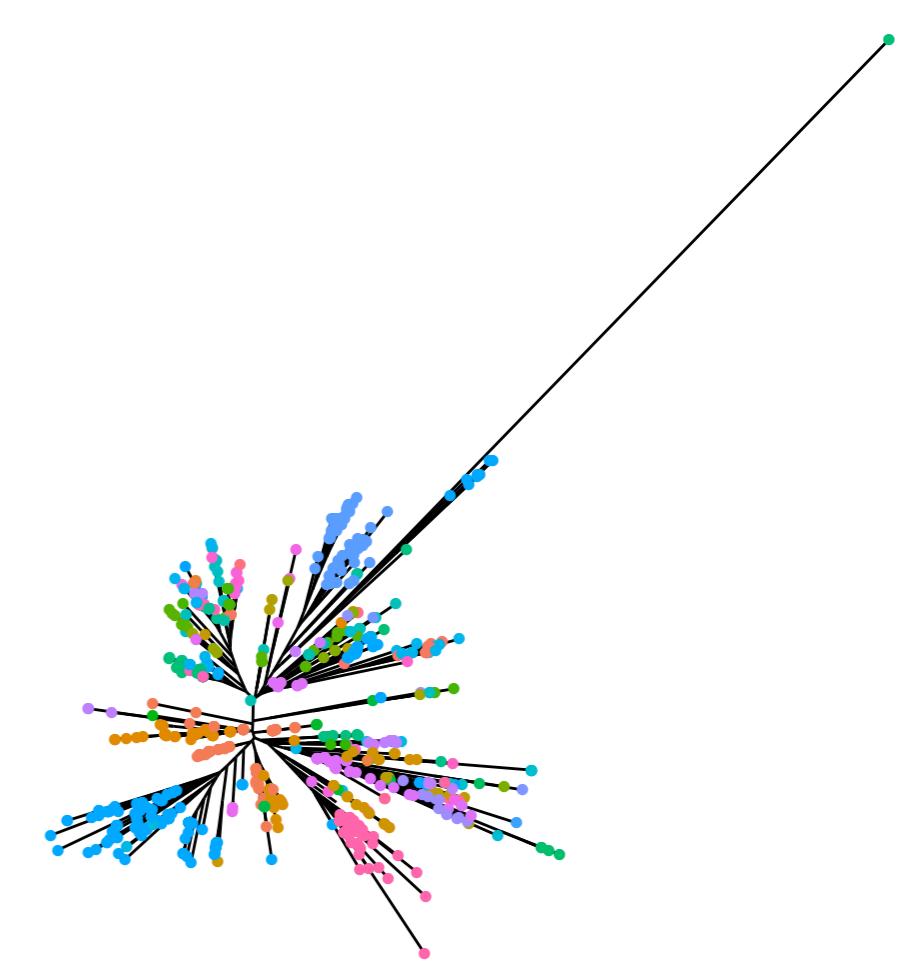
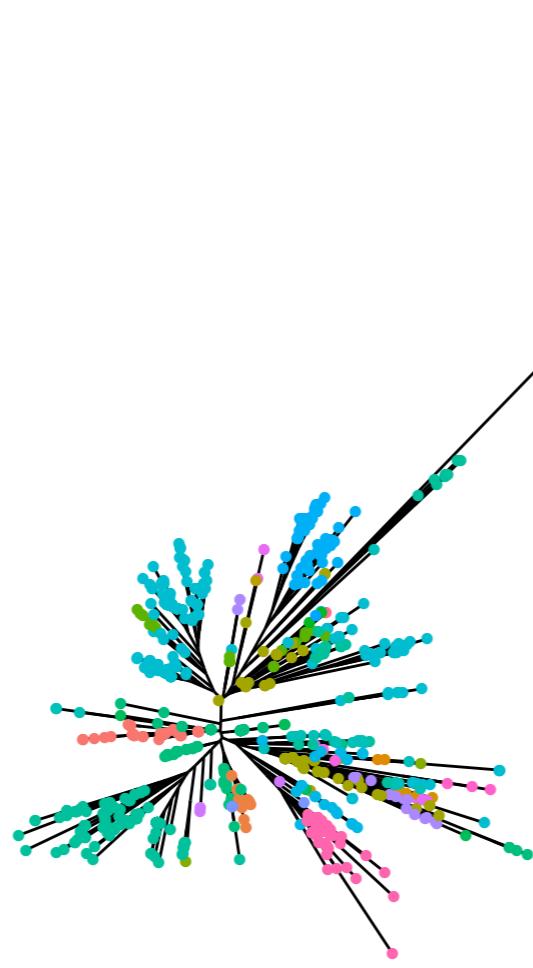
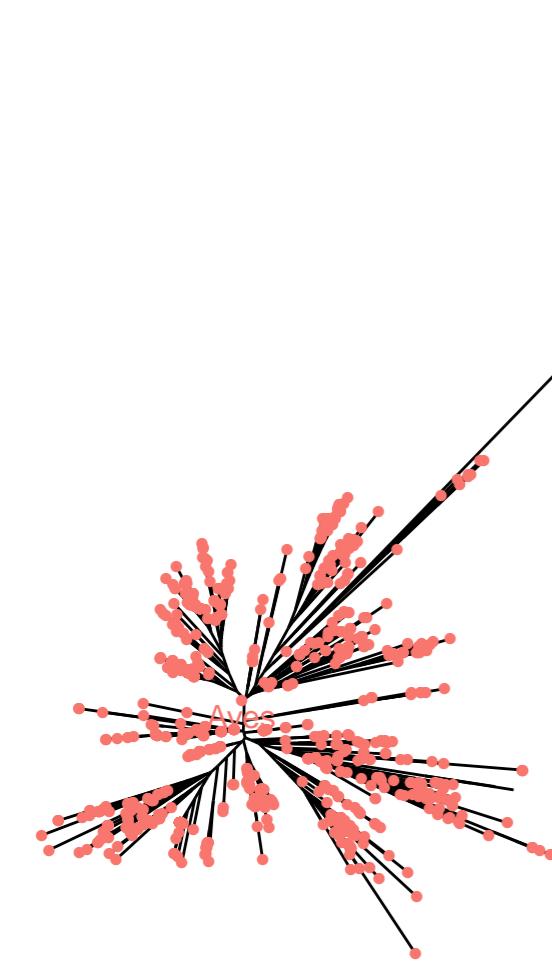
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class

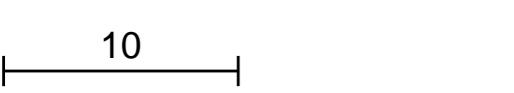
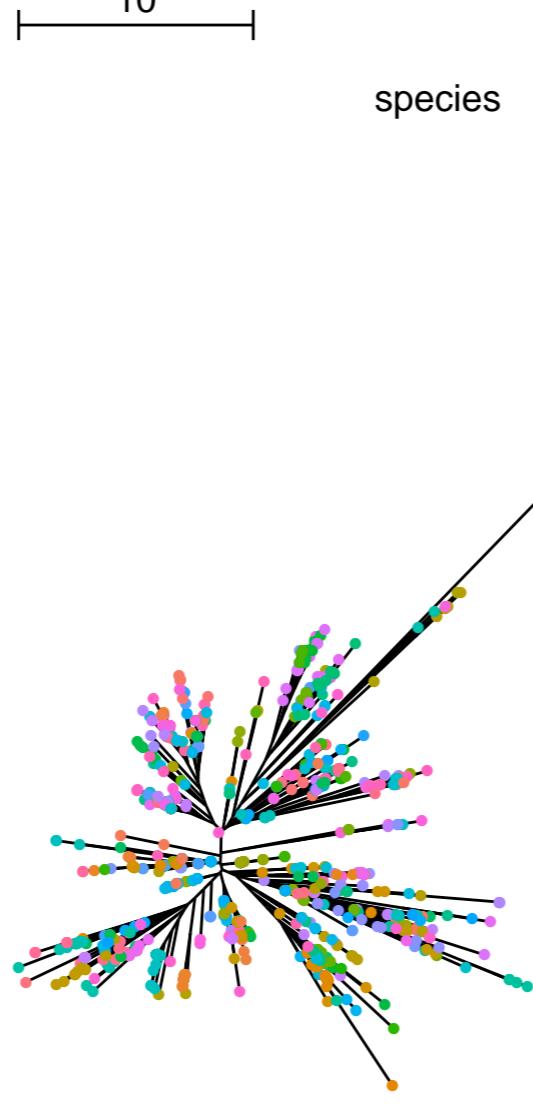
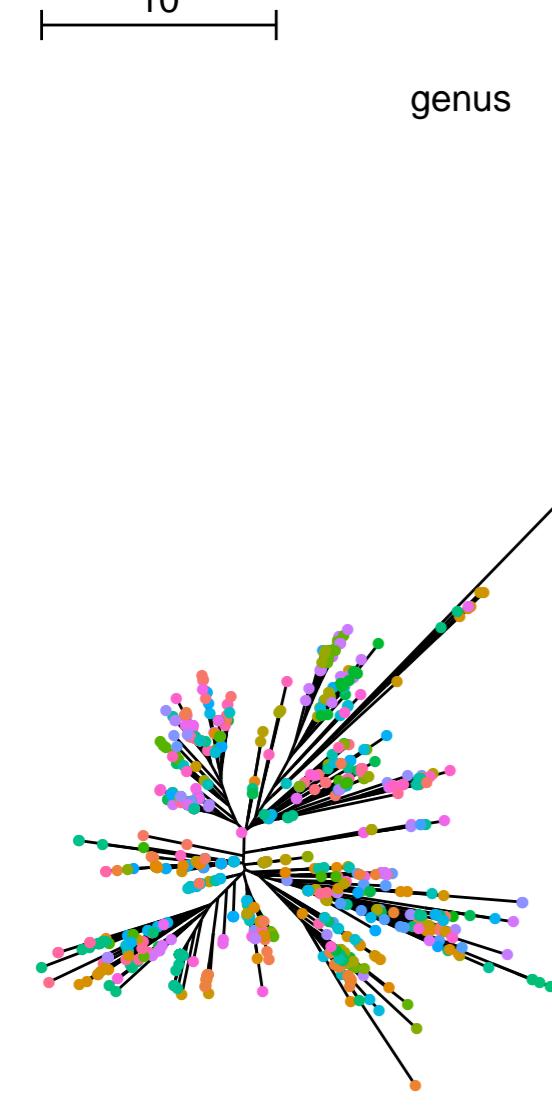
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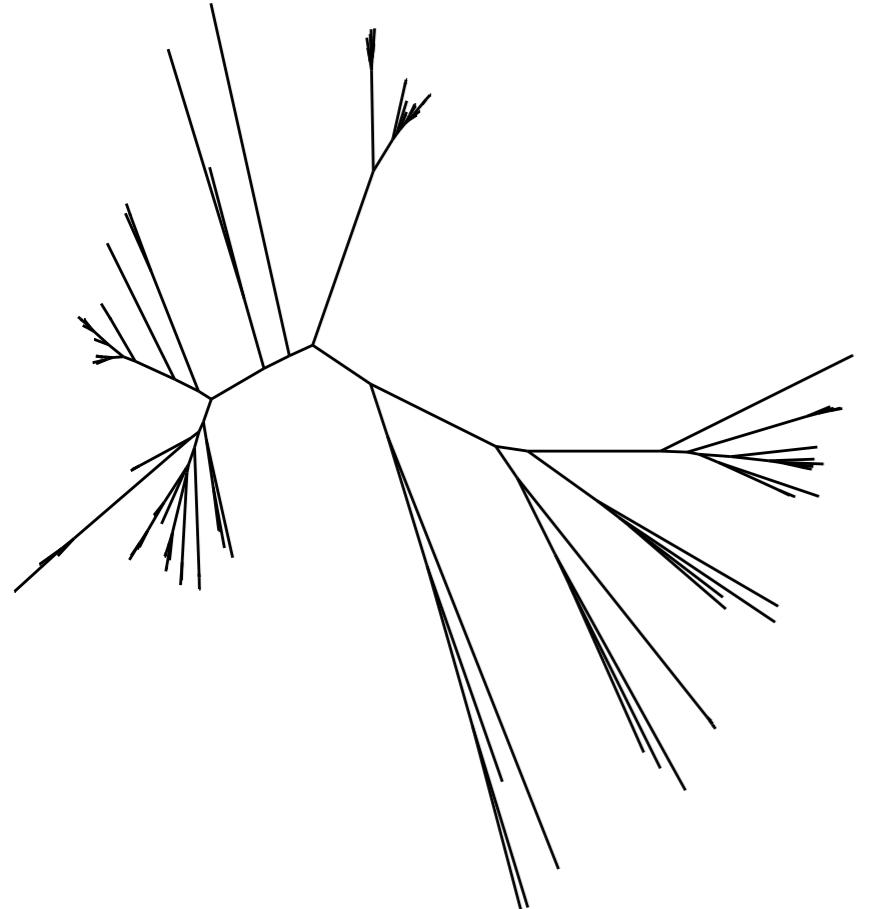


genus

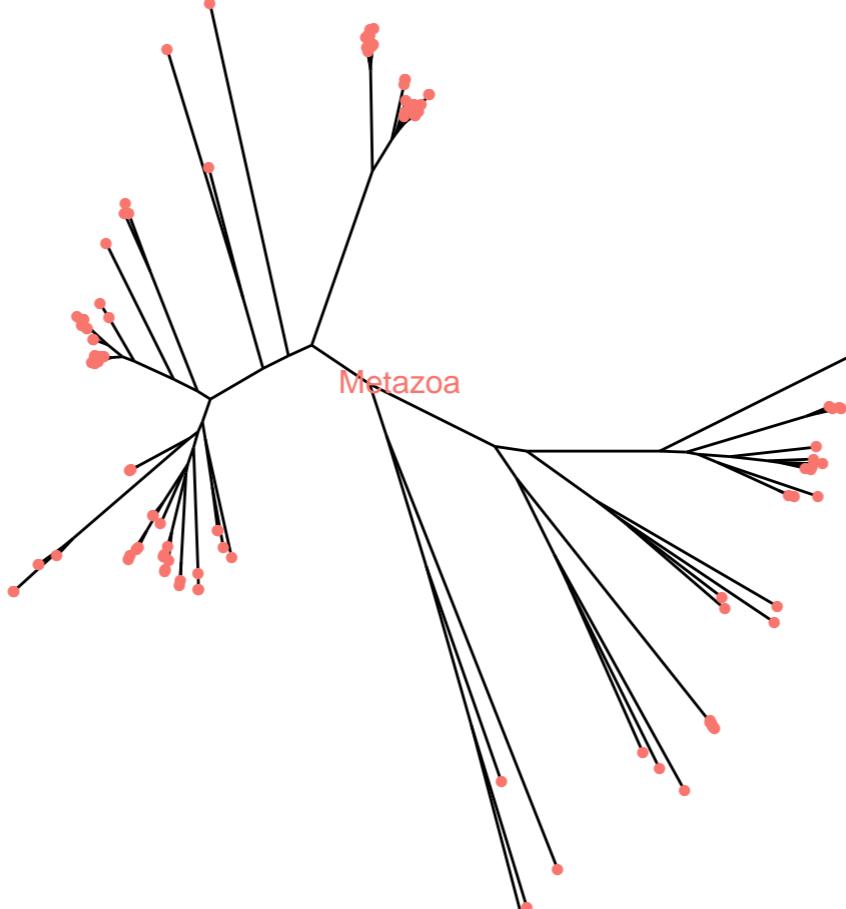
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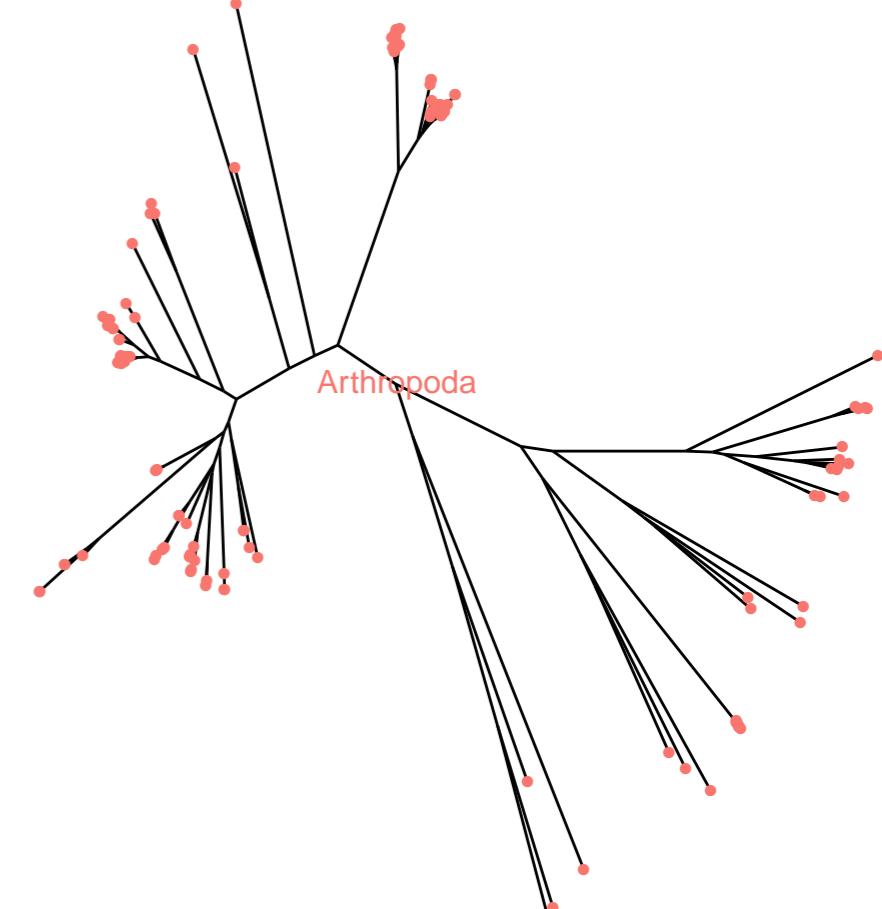
Copepod 28S



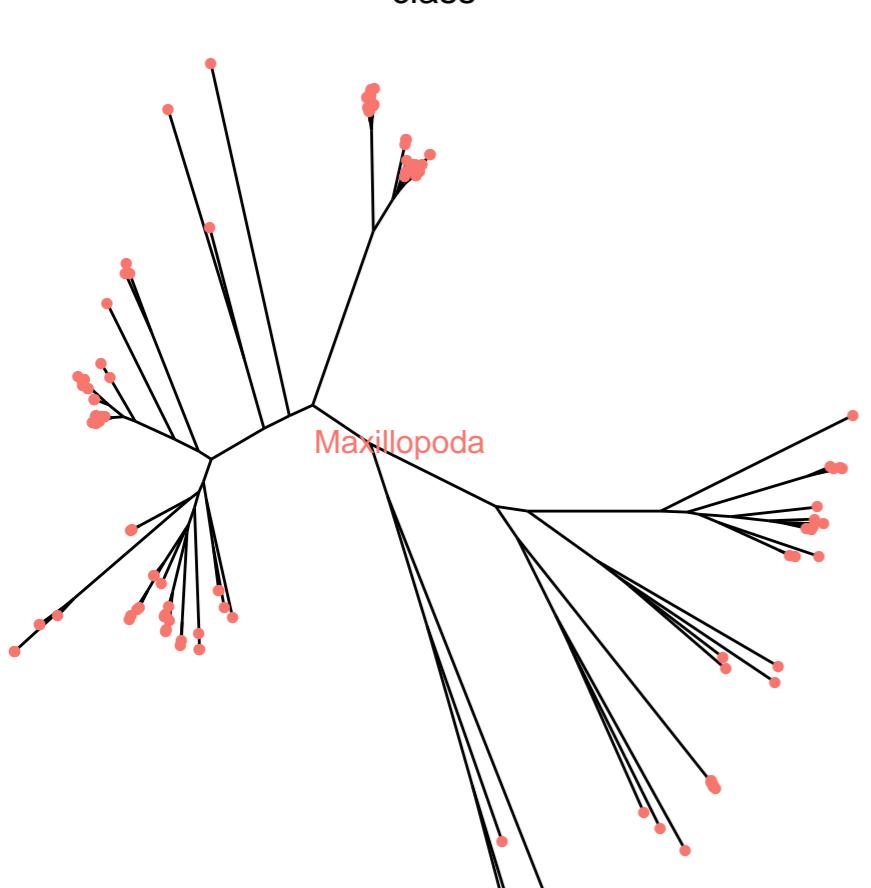
kingdom



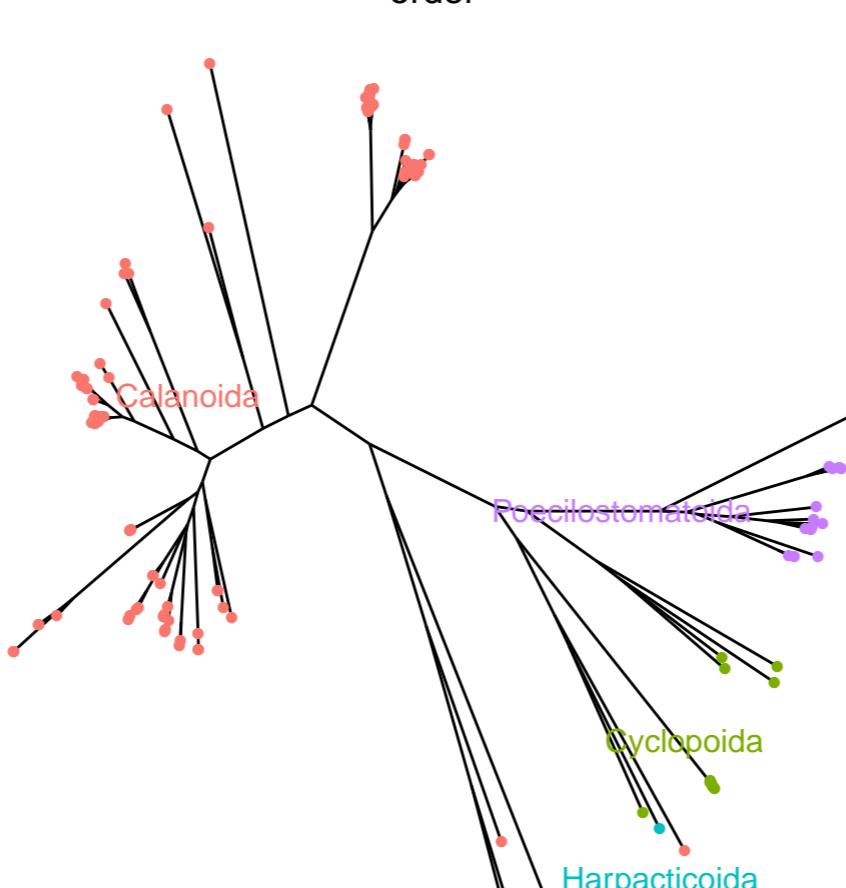
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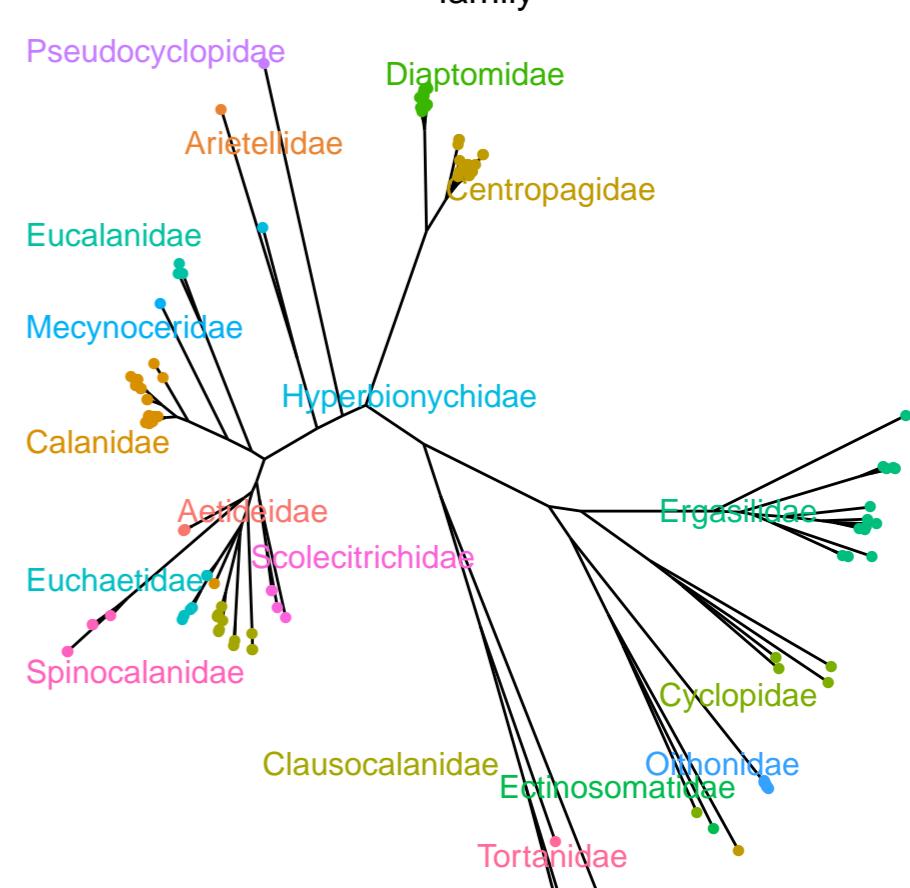
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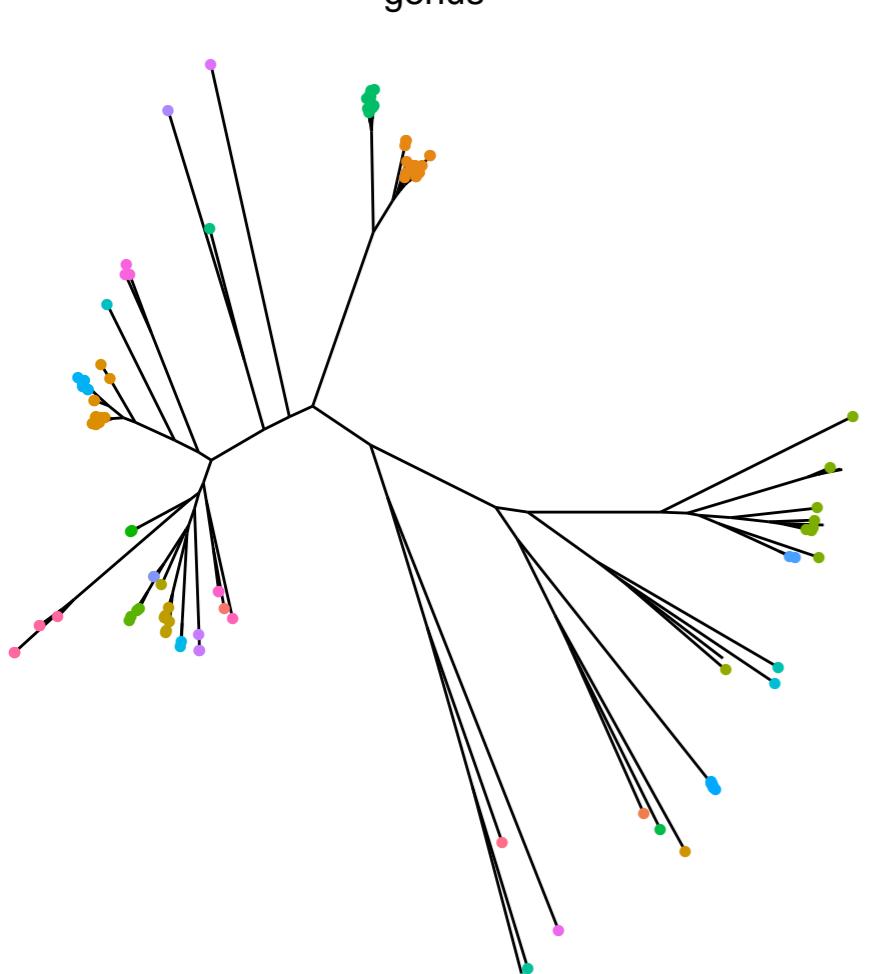
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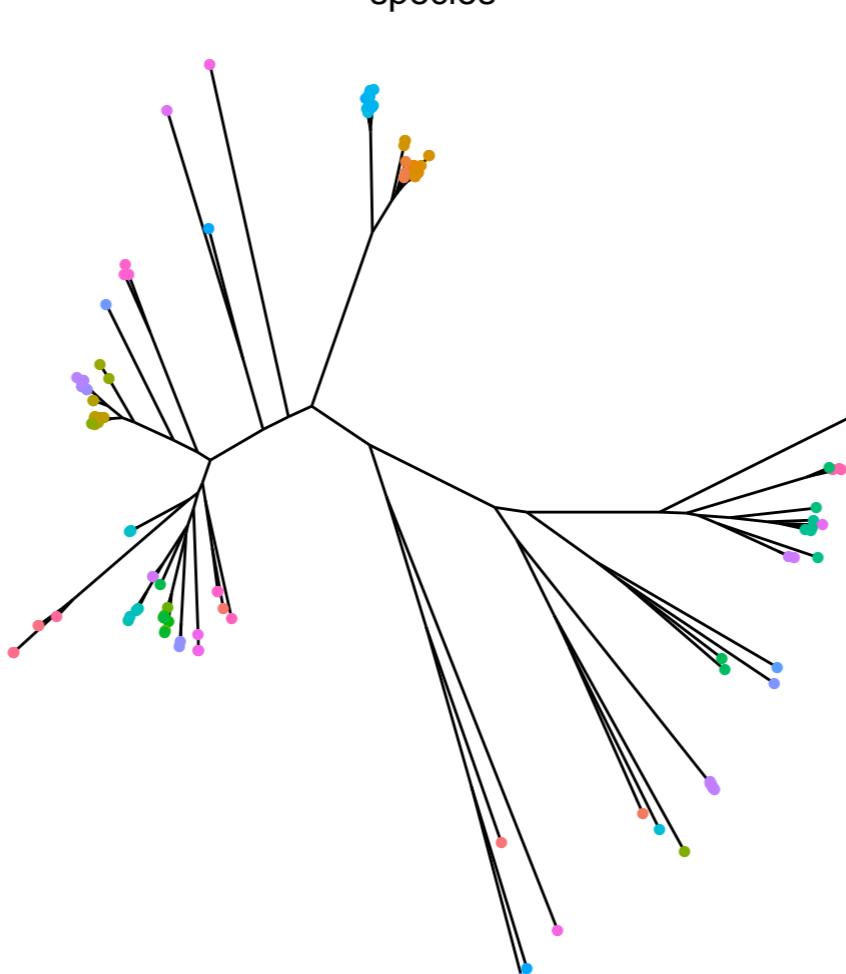
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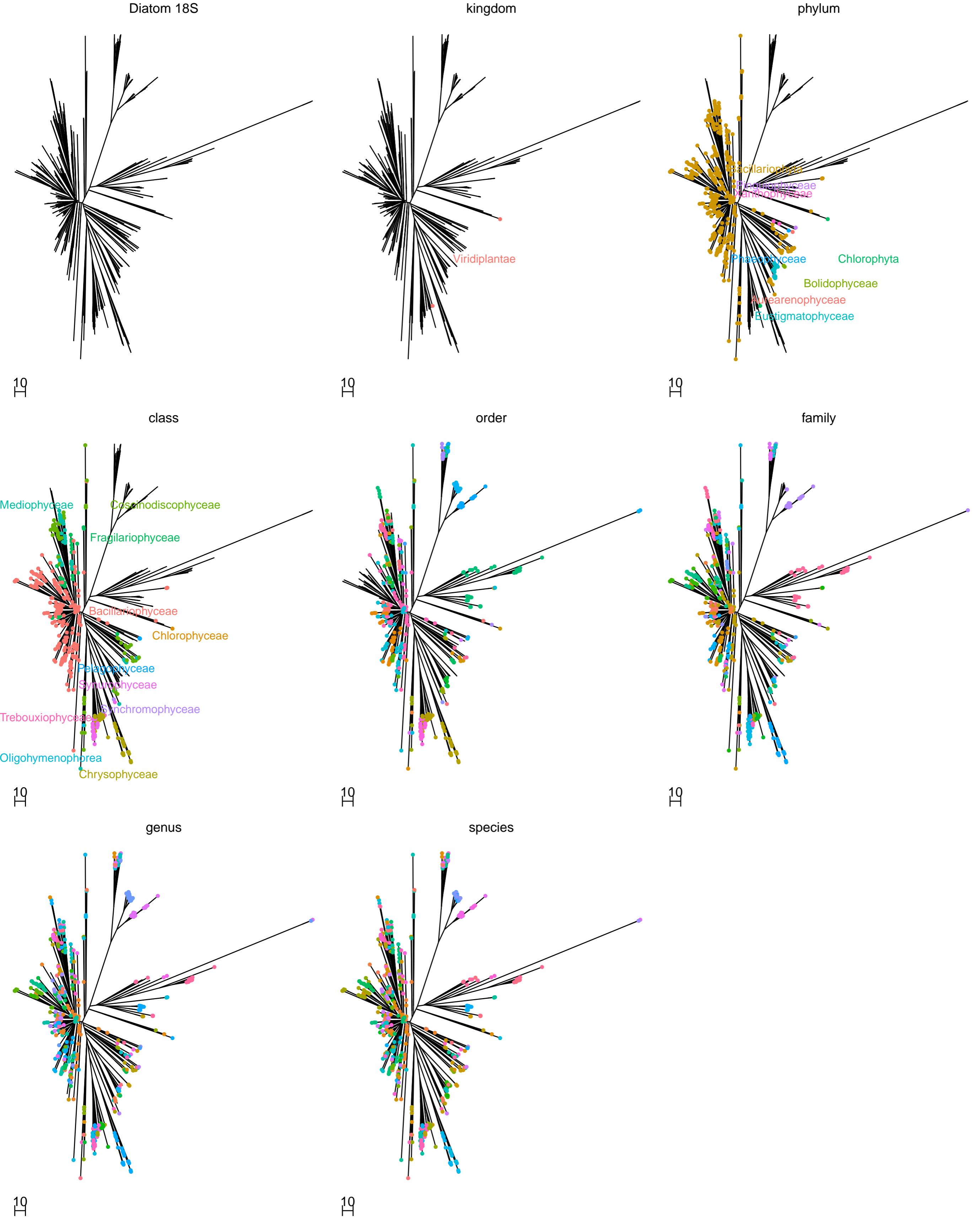


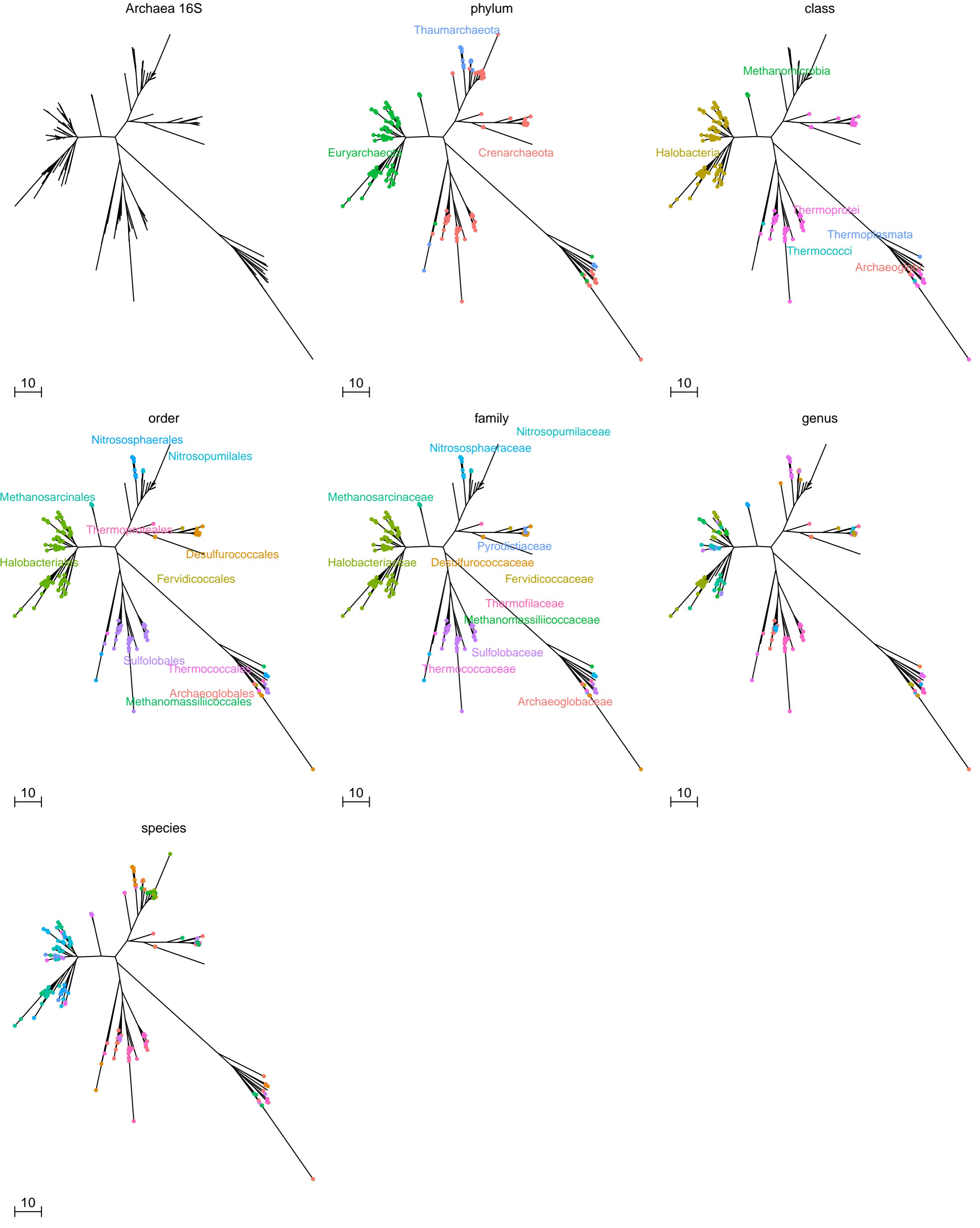
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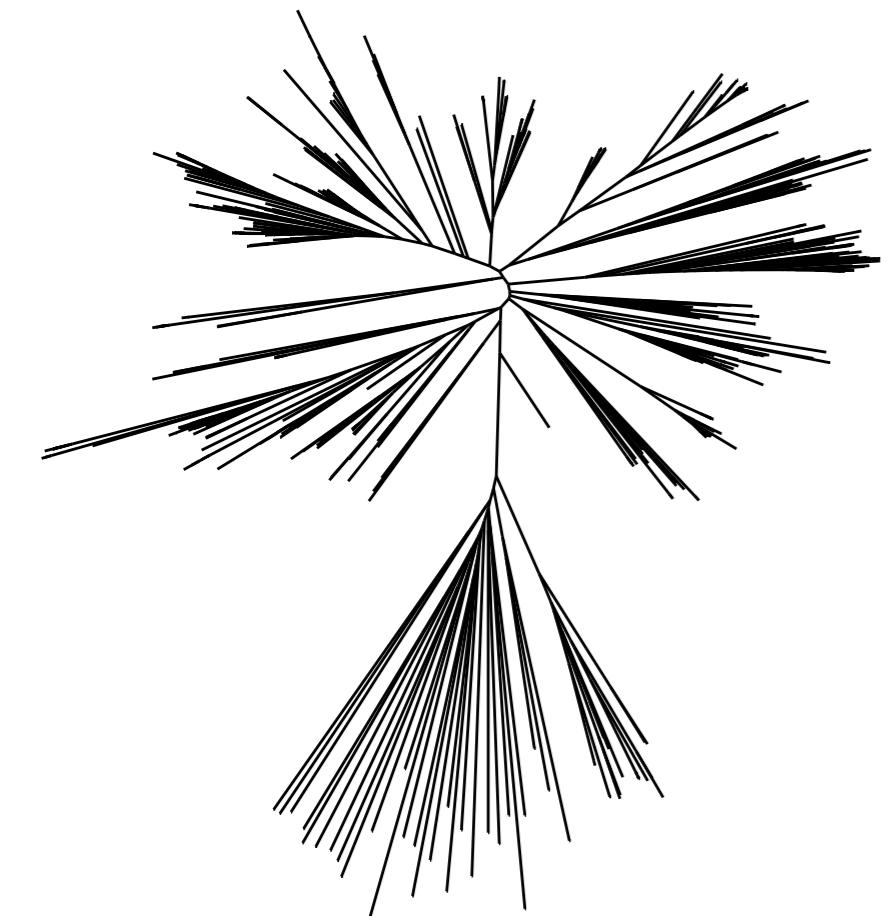
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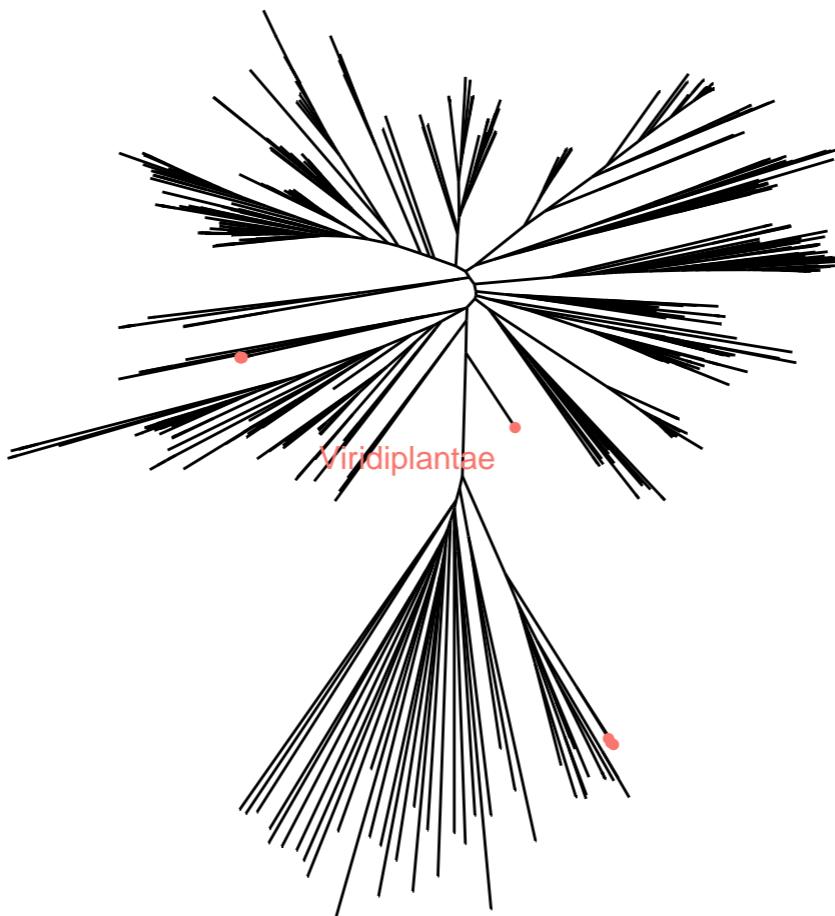




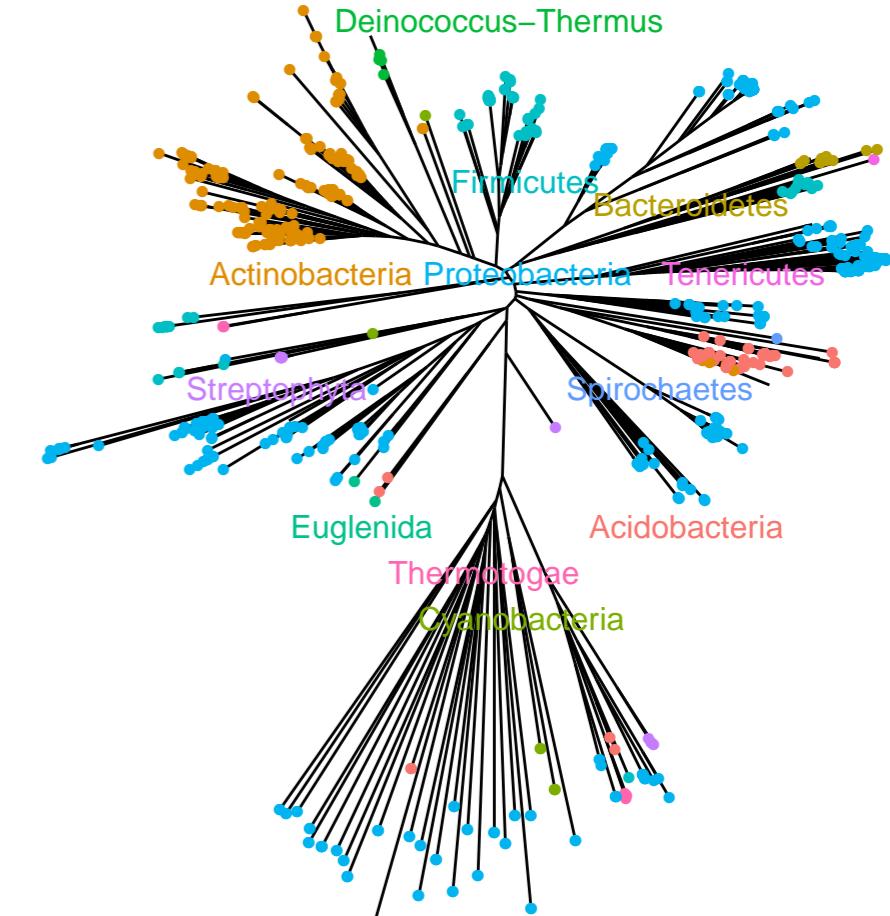
Bacteria 16S



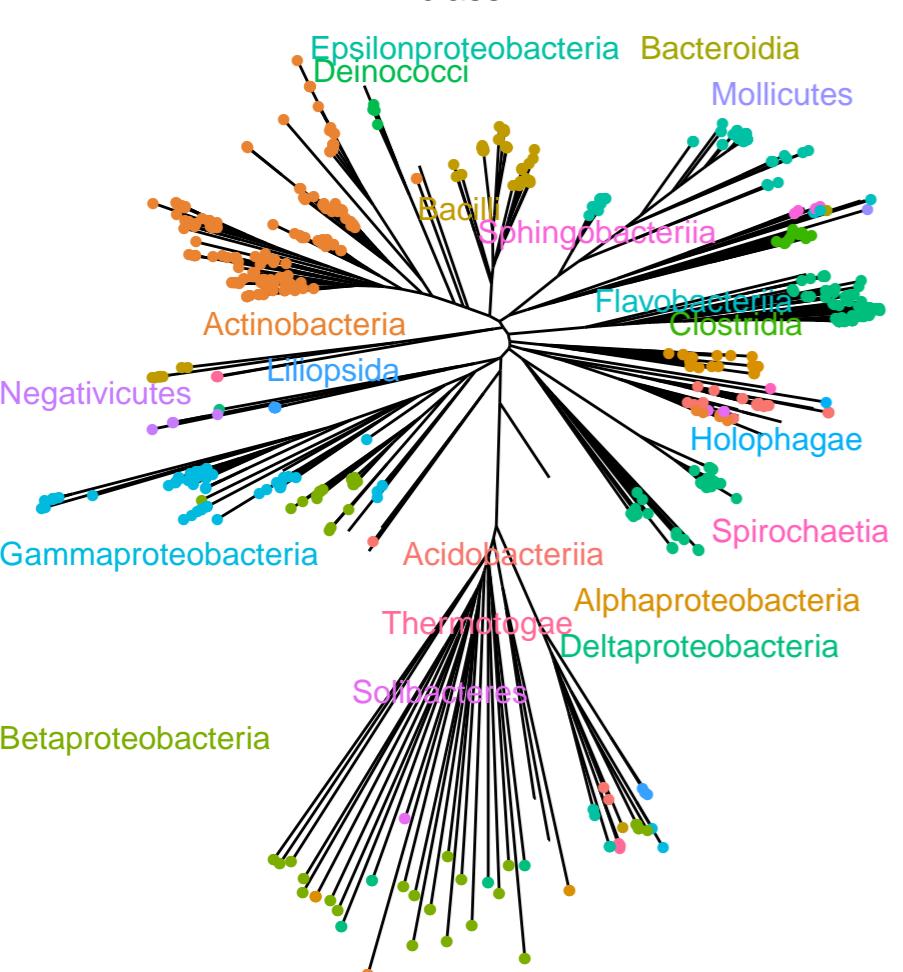
kingdom



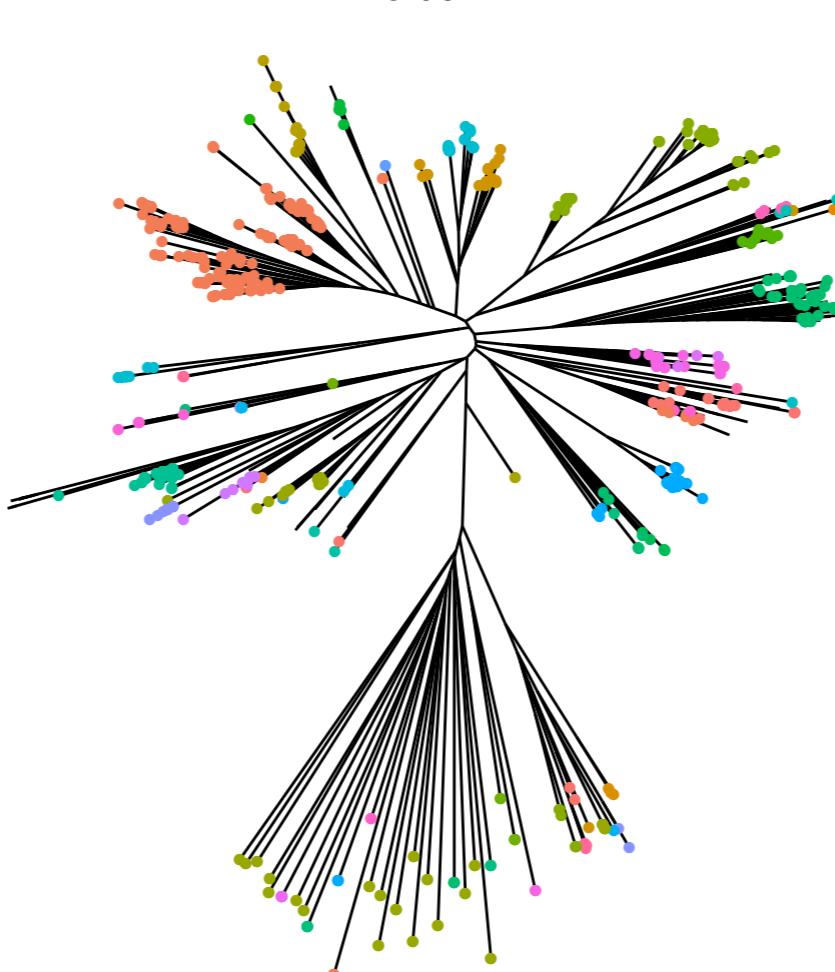
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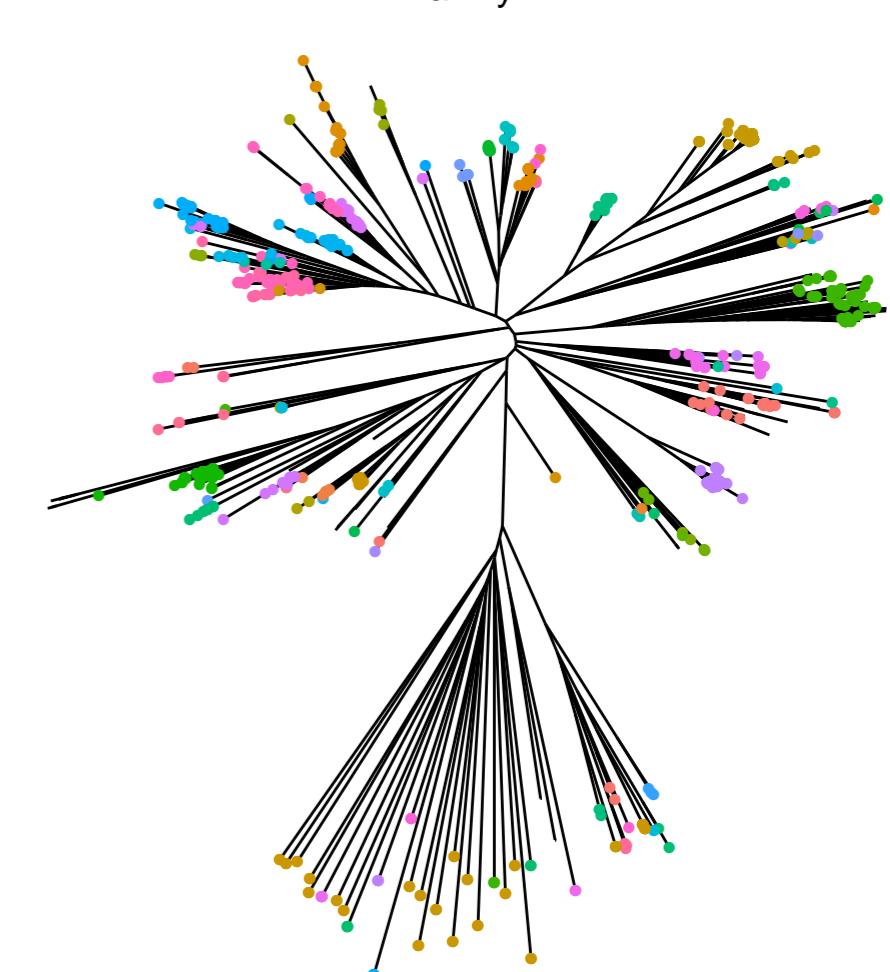
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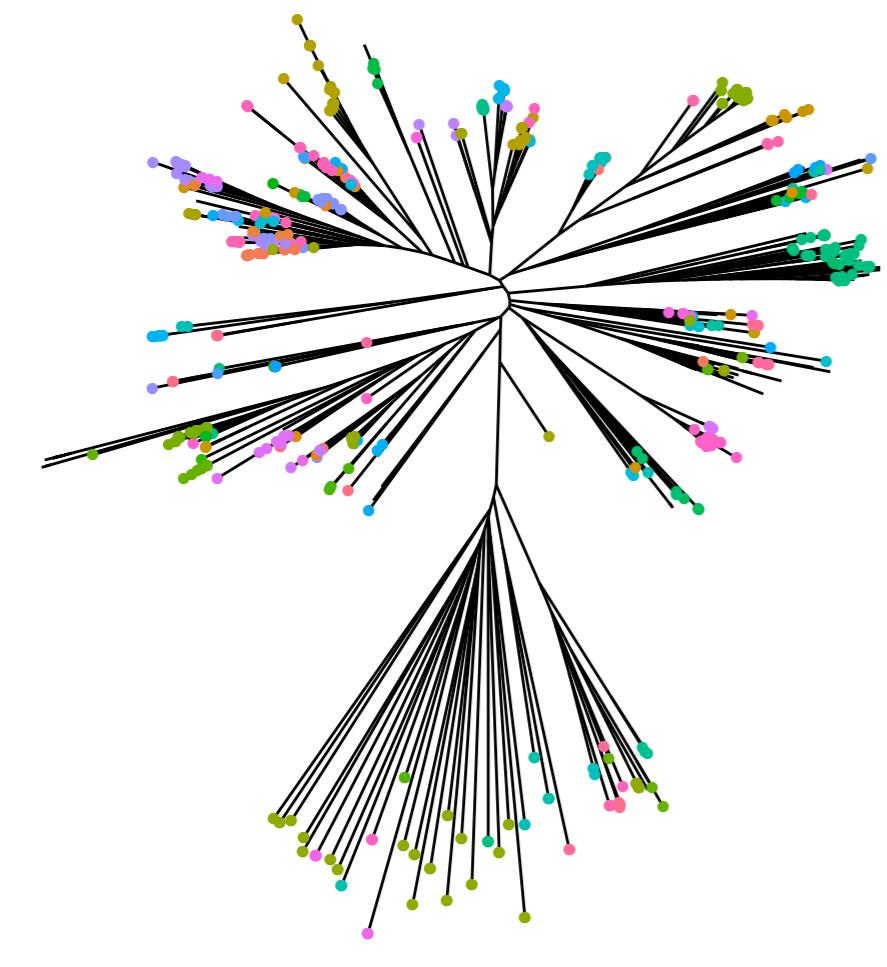
order



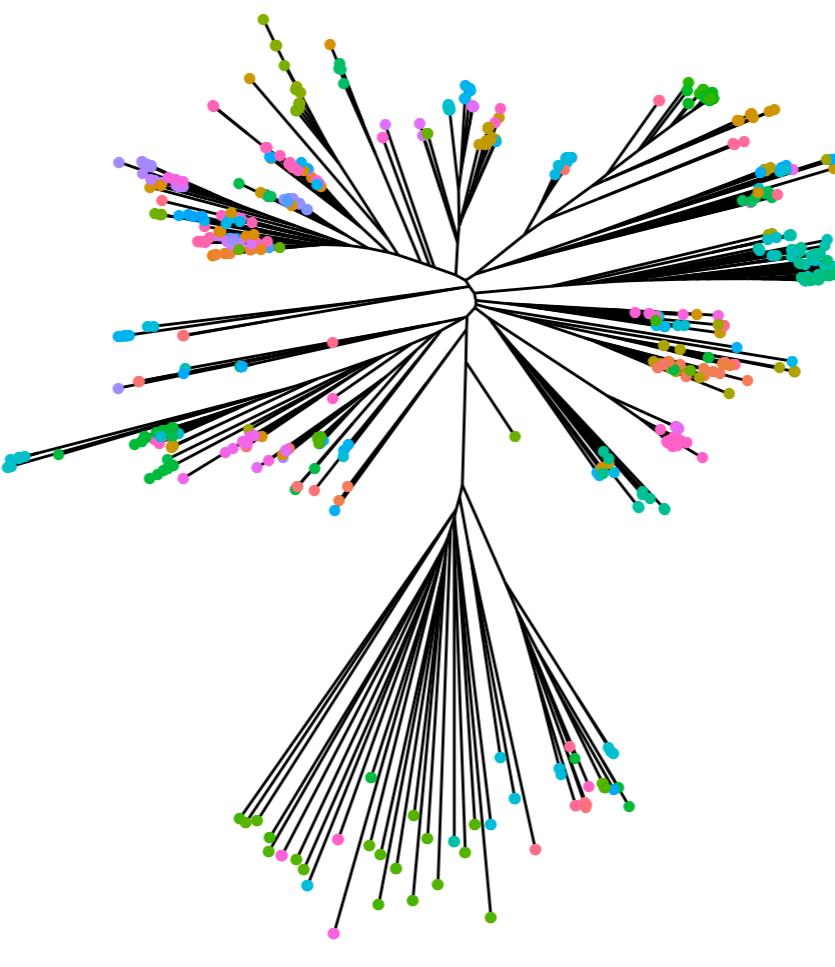
family



genus



species



10

10

Supplemental Table 1

ID	Day	Sample	LAT	LON	Notes	Time	Portion	Rain	InRiver
1	1	DS92	41.38616793	-81.15735861		8:11 AM	Upper	Before	yes
2	1	DS24	41.38289094	-81.15737203		8:26 AM	Upper	Before	yes
3	1	DS80	41.38242415	-81.15462989		8:36 AM	Upper	Before	yes
4	1	DS39	41.37719879	-81.15318334		8:50 AM	Upper	Before	yes
5	1	DS85	41.37009471	-81.16215114		9:05 AM	Upper	Before	yes
6	1	DS28	41.3629835	-81.16263611		9:22 AM	Upper	Before	yes
7	1	DS1	41.34894742	-81.16448415		9:39 AM	Upper	Before	yes
8	1	DS63	41.34008037	-81.16737968		9:50 AM	Upper	Before	yes
9	1	DS66	41.32691433	-81.17250907		10:13 AM	Upper	Before	yes
10	1	DS87	41.31662386	-81.18341435		10:38 AM	Upper	Before	yes
11	1	DS74	41.31437793	-81.19217578	Brook coming out of the culvert	10:51 AM	Upper	Before	no
12	1	DS86	41.31015153	-81.1946922		11:02 AM	Upper	Before	yes
13	1	DS23	41.30765162	-81.1960903	Water from pipe	11:08 AM	Upper	Before	no
14	1	DS61	41.30293823	-81.20072516		11:24 AM	Upper	Before	no
15	1	DS22	41.30293823	-81.20072516			Upper	Before	yes
16	1	DS5	41.30293823	-81.20072516	Pond next to river	11:26 AM	Upper	Before	no
17	1	DS38	41.2907584	-81.21081403		11:43 AM	Upper	Before	yes
18	1	DS25	41.28487037	-81.21658481	Retention spill - outside/river	11:56 AM	Upper	Before	yes
19	1	DS69	41.28487037	-81.21658481	Retention spill - inside		Upper	Before	yes
21	1	DS81	41.28268839	-81.21983078	Drainage pipe	12:03 PM	Upper	Before	no
22	1	DS88	ND	ND	100 m downstream of 21	12:05 PM	Upper	Before	yes
23	1	DS32	41.2797217	-81.22239229		12:12 PM	Upper	Before	yes
24	1	DS58	41.27846492	-81.22087961	Brook	12:18 PM	Upper	Before	no
25	1	DS89	ND	ND	WWTP Mantua	12:27 PM	Upper	Before	yes
26	1	DS15	41.27651059	-81.22707878		12:31 PM	Upper	Before	yes
27	1	DS10	41.27373467	-81.23310386		12:40 PM	Upper	Before	yes
28	1	DS52	41.26907567	-81.24587134		12:58 PM	Upper	Before	yes
29	1	DS75	41.25905394	-81.25497258		1:13 PM	Upper	Before	yes
30	1	DS94	41.25213861	-81.26298711		1:29 PM	Upper	Before	yes
31	1	DS90	41.24900957	-81.28321608		1:47 PM	Upper	Before	yes
32	1	DS72	41.12303434	-81.51505256		3:36 PM	Middle	Before	yes
33	1	DS83	41.12168653	-81.51962757		4:03 PM	Middle	Before	yes
34	1	DS6	41.11745937	-81.52206712		4:25 PM	Middle	Before	yes
35	1	DS67	41.11906174	-81.52647978		4:40 PM	Middle	Before	yes
36	1	DS27	41.12984237	-81.5381261		4:57 PM	Middle	Before	yes
37	1	DS36	41.13340736	-81.54780149	Double-drain	5:10 PM	Middle	Before	yes
38	1	DS37	41.13783586	-81.55639571		5:21 PM	Middle	Before	yes
39	1	DS62	ND	ND	100 m downstream 38, near drain	5:25 PM	Middle	Before	yes
40	1	DS13	41.14409513	-81.56314046		5:38 PM	Middle	Before	yes
41	1	DS91	41.15556702	-81.57106698	Sewer plant	5:49 PM	Middle	Before	yes
42	1	DS53	41.15980189	-81.57297227	WPCS Akron Waterfall	5:56 PM	Middle	Before	no
43	1	DS55	ND	ND	Flooded river in morning		Middle	After	yes
1	2	DS77	41.123059	-81.515063		10:34 AM	Middle	After	yes
2	2	DS50	41.117976	-81.524711		11:43 AM	Middle	After	yes
3	2	DS47	41.136665	-81.547141		11:59 AM	Middle	After	yes
5	2	DS18	41.181709	-81.58388		12:18 PM	Middle	After	yes
6	2	DS49	41.241662	-81.548578			Middle	After	yes
7	2	DS65	41.262888	-81.560156		12:49 PM	Middle	After	yes
10	2	DS46	41.392993	-81.629411			Lower	After	yes
12	2	DS51	41.414974	-81.638878		2:00 PM	Lower	After	yes
13	2	DS64	41.416104	-81.639279		2:06 PM	Lower	After	yes
14	2	DS14	41.417085	-81.645042	From covered drain	2:13 PM	Lower	After	no
15	2	DS4	41.419943	-81.654479	From large affluent of clear water from WWTP	2:19 PM	Lower	After	no
16	2	DS60	41.420158	-81.657798		2:26 PM	Lower	After	yes
17	2	DS84	41.422185	-81.663617	Drain LB	2:34 PM	Lower	After	no
18	2	DS26	41.42701	-81.666138	LB before suspended pipelines	2:42 PM	Lower	After	yes
19	2	DS21	41.434384	-81.664195	Near Sunoco Refinery	2:52 PM	Lower	After	yes
20	2	DS57	41.439323	-81.669733		3:03 PM	Lower	After	yes
21	2	DS41	41.443194	-81.677473		3:09 PM	Lower	After	yes

22_out	2 DS7	41.444157	-81.677299	Pipe + outside retention line	3:13 PM	Lower	After	yes
22_in	2 DS34	41.444157	-81.677299	Pipe + inside retention line		Lower	After	yes
23	2 DS54	41.448046	-81.684159		3:25 PM	Lower	After	yes
24	2 DS2	41.449179	-81.681288	ArcelorMittal Outfall	3:28 PM	Lower	After	no
25	2 DS35	41.460835	-81.683129		3:47 PM	Lower	After	yes
26	2 DS19	41.464189	-81.679458	Drain from metal basin	3:52 PM	Lower	After	no
27	2 DS40	41.467604	-81.671395		4:04 PM	Lower	After	yes
28	2 DS70	41.475447	-81.670943		4:23 PM	Lower	After	yes
29	2 DS9	41.481574	-81.675583		4:32 PM	Lower	After	yes
30	2 DS3	41.485641	-81.678992	Shelly Liquid terminal	4:42 PM	Lower	After	yes
31	2 DS45	41.489249	-81.684129		4:50 PM	Lower	After	yes
32	2 DS71	41.487623	-81.693352		5:06 PM	Lower	After	yes
33	2 DS78	41.493653	-81.692506		5:13 PM	Lower	After	yes
34	2 DS12	41.488853	-81.703449		5:31 PM	Lower	After	yes
35	2 DS95	41.495575	-81.702833		5:43 PM	Lower	After	yes
36	2 DS43	41.500043	-81.708604		5:59 PM	Lower	After	yes
37	2 DS82	41.503674	-81.712785	In harbor, end of pier before Wendy Park	6:02 PM	Lower	After	yes
1 M	DS33	41.4252	-81.15651667			Upper	After	yes
2 M	DS20	41.41858333	-81.15888333			Upper	After	yes
3 M	DS17	41.4125	-81.15595			Upper	After	yes
4 M	DS68	41.40428333	-81.16113333			Upper	After	yes
5 M	DS11	41.39693333	-81.15885			Upper	After	yes
6 M	DS79	41.3908	-81.15976667			Upper	After	yes
7 M	DS31	41.38608333	-81.15741667			Upper	After	yes
8 M	DS42	41.38158333	-81.1584			Upper	After	yes
9 M	DS30	41.37728333	-81.15076667			Upper	After	yes
10 M	DS76	41.37473333	-81.1563			Upper	After	yes
11 M	DS73	41.36871667	-81.15995			Upper	After	yes
12 M	DS93	41.3609	-81.1626			Upper	After	yes
13 M	DS59	41.35051667	-81.1637			Upper	After	yes
14 M	DS8	41.33803333	-81.16733333			Upper	After	yes
15 M	DS48	41.32778333	-81.17135			Upper	After	yes

Supplemental Table 2

Primer	Forward Primer	Reverse Primer	Target	Citation
trNL	CTTTCCCTACAGCACGCTTCCGATCTGGCAATCTGGCAA	GTGACTGGAGTCAGCGTGTCTCCGATCTCCATTGAGTCCTGCACCTATC	Vascular plants	Taberlet et al. 2007
16Smm	CTTTCCCTACAGCACGCTTCCGATCTGGTGGGTCACCTGGA	GTGACTGGAGTCAGACGCTGTCCTCCGATCTGTTATCCCCTAGGTAAC	Mammals	Taylor, 1996
COI_ZBJ_Art	CTTTCCCTACAGCACGCTTCCGATCTAGATATTGGAACWT	GTGACTGGAGTCAGACGCTGTCCTCCGATCTWAATACTWCCAATCTCC	Insects+Arachn	Zeale et al., 2011
23SrDNA	CTTTCCCTACAGCACGCTTCCGATCTGGACAGAAAGCCATGAA	GTGACTGGAGTCAGACGCTGTCCTCCGATCTTGACAGGCTTC	Algae	Sherwood and Presting, 2007
AmpCB	CTTTCCCTACAGCACGCTTCCGATCTGGTGGGTCACCTGGA	GTGACTGGAGTCAGACGCTGTCCTCCGATCTGTTATCCCCTAGGTAAC	Amphibians	Thomsen et al., 2012
FungITS	CTTTCCCTACAGCACGCTTCCGATCTGGAAAGCTGAAAGG	GTGACTGGAGTCAGACGCTGTCCTCCGATCTAAAGAGATCCGTTGAAAGTT	Fungi	Epp et al., 2012
BryoTrnL	CTTTCCCTACAGCACGCTTCCGATCTGGAAAGCTGAAAGG	GTGACTGGAGTCAGACGCTGTCCTCCGATCTCAATGAGTCCTGACC	Bryophytes	Epp et al., 2012
Aves125	CTTTCCCTACAGCACGCTTCCGATCTGGATTAGATACCCACTATGC	GTGACTGGAGTCAGACGCTGTCCTCCGATCTGGGCTGACTCTCG	Birds	Epp et al., 2012
Cop285	CTTTCCCTACAGCACGCTTCCGATCTGGTGTGAAACCGGAG	GTGACTGGAGTCAGACGCTGTCCTCCGATCTGGGCTGACTCTCG	Copepod	Bisset et al., 2005
Diatom185	CTTTCCCTACAGCACGCTTCCGATCTTCGACTTCAAGCTTACGG	GTGACTGGAGTCAGACGCTGTCCTCCGATCTGTTAACCGTGTAACT	Diatom	Zimmerman et al., 2011
FishCB	CTTTCCCTACAGCACGCTTCCGATCTTCGACTTGGGGCTACAGT	GTGACTGGAGTCAGACGCTGTCCTCCGATCTGGAAAGATCTGTT	Fish	Thomsen et al., 2012
Archaea16S	CTTTCCCTACAGCACGCTTCCGATCTCCGACGCTGAGRGRYGA	GTGACTGGAGTCAGACGCTGTCCTCCGATCTGCTCCCCGCCAATTCCT	Archaea	Baker et al., 2003
Barcodeing Primer	Primer	Barcode		
DS01	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AACCGC		
DS02	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AACGCC		
DS03	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AAGCGG		
DS04	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AAGGGC		
DS05	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACACAG		
DS06	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACACTC		
DS07	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACAGAC		
DS08	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACAGTG		
DS09	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACACAT		
DS10	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACACAGA		
DS11	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACCTCA		
DS12	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACCTGT		
DS13	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACGACA		
DS14	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACGAGT		
DS15	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACGCT		
DS16	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACGTGA		
DS17	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACTCAC		
DS18	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACTCTG		
DS19	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACTGAG		
DS20	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ACTGTC		
DS21	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGACAC		
DS22	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGACTG		
DS23	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGAGAG		
DS24	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGAGTC		
DS25	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGCACA		
DS26	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGCAGT		
DS27	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGCTCT		
DS28	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGCTGA		
DS29	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGGACT		
DS30	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGGAGA		
DS31	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGGCTA		
DS32	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGGTGT		
DS33	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGTCAG		
DS34	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGTCTC		
DS35	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGTGCAC		
DS36	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	AGTTGT		
DS37	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ATCCGG		
DS38	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ATCCGG		
DS39	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ATCGGC		
DS40	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ATGCCG		
DS41	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ATGGCC		
DS42	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	ATGGCC		
DS43	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CAACCT		
DS44	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CAACGA		
DS45	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CAAGCA		
DS46	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CAAGGT		
DS47	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CACAG		
DS48	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CACATC		
DS49	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CACTAC		
DS50	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CACTTG		
DS51	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CAGAAC		
DS52	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CAGATG		
DS53	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CAGTAG		
DS54	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CAGTTC		
DS55	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CATCCA		
DS56	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CATCGT		
DS57	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CATGCT		
DS58	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CATGGA		
DS59	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCAACG		
DS60	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCAAGC		
DS61	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCATCC		
DS62	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCATGG		
DS63	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCGCAA		
DS64	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCGCTT		
DS65	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCGGAT		
DS66	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCGGTA		
DS67	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCCTAC		
DS68	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCTAGG		
DS69	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCTTCG		
DS70	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CCTTGC		
DS71	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGAAC		
DS72	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGAAAG		
DS73	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGATCG		
DS74	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGATGC		
DS75	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGCCAA		
DS76	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGCCTT		
DS77	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGCGAT		
DS78	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGCGTA		
DS79	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGGCAT		
DS80	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGGCTA		
DS81	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGTAGC		
DS82	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGTAGC		
DS83	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGTCCC		
DS84	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CGTTGG		
DS85	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CTACCA		
DS86	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CTAGCT		
DS87	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CTAGCT		
DS88	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CTAGGA		
DS89	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CTCAAC		
DS90	CAAGCAGAACAGCGCATACGAGATAACCGCGTACTGGAGTTC	CTCATG		

DS91	CAAGCAGAAGACGGCATACGAGATCTTAGGTGACTGGAGTTC	CTCTAG
DS92	CAAGCAGAAGACGGCATACGAGATCTTICGTGACTGGAGTTC	CTCTTC
DS93	CAAGCAGAAGACGGCATACGAGATCTGAAGGTGACTGGAGTTC	CTGAAG
DS94	CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTC	CTGATC
DS95	CAAGCAGAAGACGGCATACGAGATCTGACGTGACTGGAGTTC	CTGTAC
DS96	CAAGCAGAAGACGGCATACGAGATCTGGGTGACTGGAGTTC	CTGTTG
Barcode primer R	AATGATACGGCGACCACCGAGATCTACACTTTCCCTACACGAC	

Supplemental Table 3

Primer	Mean Length (range)	Kingdom	Phylum	Class	Order	Family	Genus	Species	# Unique sequences
16SArchaea	152 bp (51 - 288)	1.00 (1.00)	1.00 (1.14)	1.02 (1.14)	1.02 (1.14)	1.17 (1.22)	3.13 (1.84)	3.60 (8.73)	3029
16Smam	95 bp (76 - 118)	1.00 (1.01)	1.03 (1.01)	1.03 (1.01)	1.00 (1.01)	1.03 (1.01)	1.06 (1.10)	1.27 (1.46)	727
23SrDNA	334 bp (65 - 341)	1.00 (1.01)	1.00 (1.02)	1.07 (1.04)	1.06 (1.31)	1.14 (1.57)	1.25 (2.06)	1.34 (3.64)	2944
AmpCB	68 bp (68 - 68)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.01)	1.00 (1.14)	20
Aves12S	53 bp (50 - 211)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.01 (1.01)	1.41 (1.05)	2.99 (1.26)	12.39 (1.57)	134
BryoTrnL	52 bp (25 - 55)	1.00 (1.00)	1.01 (1.00)	1.01 (1.00)	1.03 (1.05)	2.13 (1.39)	9.78 (2.12)	20.07 (3.74)	262
COI_ZBJ_Art	162 bp (156 - 166)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.15 (1.05)	1.23 (1.09)	1.27 (1.18)	26
Cop28S	209 bp (53 - 368)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.01)	1.00 (1.03)	2.04 (1.14)	50
Diatom18S	359 bp (52 - 424)	1.00 (1.00)	1.50 (1.00)	1.02 (1.01)	1.10 (1.04)	1.21 (1.05)	1.38 (1.09)	1.62 (1.55)	1882
FishCB	90 bp (76 - 192)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.01)	1.00 (1.01)	1.01 (1.08)	1.45 (1.29)	229
FungusITS	163 bp (73 - 294)	1.01 (1.01)	1.03 (1.08)	1.05 (1.10)	1.07 (1.11)	1.07 (1.12)	1.60 (1.26)	2.08 (2.64)	1527
trnL	48 bp (17 - 80)	1.00 (1.00)	1.01 (1.00)	1.04 (1.00)	1.13 (1.01)	1.21 (1.01)	9.34 (4.05)	34.89 (14.33)	3113

Supplemental Table 5

Species	Fish mt-Cytb	Mammalian 16S rRNA
Central stoneroller		✓
White sucker		✓
Grass/Black carp		✓
<i>Cyprinella spiloptera</i>		✓
Common carp	✓	✓
Cypress minnow		✓
Northern hogsucker		✓
Shiner		✓
Spotted sucker		✓
Redhorse		✓
Bluehead chub		✓
Emerald shiner		✓
Blacknose dace		✓
Bluntnose minnow		✓
Creek chub	✓	

Supplemental Table 7

Extraction (DNeasy 91 samples)	\$440.00
First PCRs (12 primers)	\$335.00
Second PCR	\$28.00
Sequencing (MiSeq 150 PE)	\$1,200.00
Total (91 samples)	\$2,003.00
Per sample	\$22.01